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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US90/05105 <b>(22) International Filing Date:</b> 13 September 1990 (13.09.90)  <b>(30) Priority data:</b> 413,332                      27 September 1989 (27.09.89)    US 571,267                      23 August 1990 (23.08.90)        US  <b>(71) Applicant:</b> ATHENA NEUROSCIENCES, INC. [US/ US]; 800F Gateway Boulevard, South San Francisco, CA 94080 (US).  <b>(72) Inventors:</b> RUBIN, Lee, L. ; 122 Funston Avenue, San Francisco, CA 94118 (US). LIAW, Chen, W. ; 80 Maria- na Court, Redwood City, CA 94062 (US). TOMASELLI, Kevin, J. ; 485 Vallejo Street, San Francisco, CA 94133 (US).		<b>(74) Agents:</b> BLECHER, Melvin et al.; 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US).  <b>(81) Designated States:</b> AT (European patent), BE (European patent), CA, CH (European patent), DE (European pa- tent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (Euro- pean patent), JP, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title:</b> COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE  <b>(57) Abstract</b>  Compositions that disrupt microvascular endothelial and epithelial cell tight junctions, and methods of use, are disclosed. Such compositions comprise agents that inhibit the binding to such cells of cell adhesion molecules. Such inhibitor agents include cell adhesion molecules, fragments of cell adhesion molecules that encompass a cell-binding domain such as HAV, and antibodies directed against cell adhesion molecules and fragments thereof. Also disclosed are drug delivery compositions comprising a therapeutic drug conjugated to an agent that disrupts cell tight junctions.		

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**COMPOSITIONS FOR CELL ADHESION INHIBITION  
AND METHODS OF USE**

This is a continuation-in-part of United States  
Serial No. 07/413,332, filed September 27, 1989.

5                    **Background of the Invention**

**Field of the Invention**

                  This invention relates to compositions that  
transiently and reversibly dissociate the blood-brain  
barrier. More particularly, the invention relates to  
10    compositions that dissociate tight junctions between  
brain capillary endothelial cells that constitute the  
physiological barrier between the general circulation  
and the brain.

**Detailed Description of Related Art**

15                The entry of drugs from the blood stream to the  
central nervous system (CNS), i.e., the brain and  
spinal cord, is restricted by the presence of high  
resistance tight junctions between brain capillary  
cells and by the apparently low rate of transport  
20    across these endothelial cells (Betz, A.L., et al.,  
Ann. Rev. Physiol., 48:241 (1986); Pardridge, W.M.,  
Ann. Rev. Pharmacol. Toxicol., 28:25 (1988)).

                  The tight junctions of the blood brain barrier  
(BBB) prevent diffusion of molecules and ions around  
25    the brain capillary endothelial cells. The only  
substances that can readily pass from the luminal core  
of the capillary to the abluminal tissues that surround  
the capillary are those molecules for which selective  
transport systems exist in the endothelial cells, as  
30    well as those compounds that are lipophilic (i.e.,  
hydrophobic). In contrast, drugs, peptides and other

molecules that are neither lipophilic nor transported by specific carrier proteins are barred from entry into the brain, or their rates of entry are too low to be useful, thereby imposing a severe limitation upon the physician's ability to treat CNS disorders pharmacologically.

The carrier-mediated transcellular transport system mentioned above may have limited usefulness for therapeutic modalities under some circumstances.

Transcytotic transport, in general, involves, first, the binding of molecules to specific carrier proteins on the surface of endothelial cells, and, second, the delivery of such molecules across the endothelial cells. Limitations on the usefulness of such a system for treatment of CNS disorders are based on the following considerations: (1) physiological carrier proteins may not function efficiently, or at all, with non-physiological drugs; (2) even where function occurs, the rate of transport of therapeutic agents will be limited by the rate of transport of the carrier; (3) the overall capacity of cerebral capillary endothelial cells to transport any therapeutic macromolecules may be simply too low to achieve therapeutic levels of certain drugs in the brain; and (4) once therapeutic macromolecules enter endothelial cells, depending on their nature, they might be delivered to any number of organelles, including lysosomes that contain a wide variety of hydrolytic enzymes. For these reasons, creating drug delivery systems that do not rely upon transcytosis will clearly be advantageous.

As tight junctions between brain capillary endothelial cells constitute a major part of the BBB, the possibility of modifying these junctions has been considered. It has been found that tight junctions,

including those of the BBB, can be disrupted by hyperosmotic solutions administered intra-arterially. For example, Polley et al., WO89/04663, published June 1, 1989, disclose the osmotic disruption of the interendothelial structure of the BBB by the intra-arterial administration of hypertonic solutions of mannitol, arabinose or glycerol as a means of introducing into the brain genetic material. Similarly, hyperosmotic solutions of urea have also been used to alter the BBB (Bowman, P.D. et al., Ped. Res., 16:335A (1982)).

Other chemical agents have been reported to disrupt endothelial or epithelial cell tight junctions when administered intravenously, including:

7-fluorouracil (MacDonell, L.A., et al., Cancer. Res., 38:2930 (1978)), degradation by membrane enzymes (Vincent, P.A., et al., Exp. Mol. Path., 48:403 (1988); Diener, H.M., et al., J. Immunol., 135:537 (1985)), aluminum salts (Zigler, Z.Y., et al., IRCS Med. Sci., 12:1095 (1984)), histamine (Meyrick, B., et al., Exp. Lung Res., 6:11 (1984)), thrombin (Siflinger-Birnboim, A., et al., Microvasc. Res., 36:216 (1988)), phorbol esters (Shiba, K., et al., Exp. Cell Res., 178:233 (1988)), and neutralization of the luminal anionic charge (Hart, M.M., J. Neuropathol. Exp. Neurol., 46:141 (1987)).

Although the above-listed modalities may disrupt tight junctions and thereby increase permeability of the BBB, problems attendant upon their use make them less than desirable. For example, intra-arterial perfusion with hyperosmotic solutions involves surgery, and this cannot be repeated on a regular basis. Further, concentrated sugar solutions may not be innocuous, and might be expected to have undesirable side effects. In addition, the aforementioned chemical

agents may not be useful for the treatment of chronic neurological disease, their effects on tight junctions are not always reversible, and, as they all are themselves powerful drugs, there is always the danger that their use will compromise the patient's health generally. For example, 7-fluorouracil is a powerful inhibitor of pyrimidine synthesis, and thus nucleic acid biosynthesis, in animals cells.

Thus, an important need still exists for means which transiently and reversibly disrupt tight junctions of the BBB in order that administered drugs can reach the brain from the general circulation, and which have no undesirable side effects of their own in the subject.

Attempts have been made to disrupt cell-cell adhesion by modifying the protein(s) responsible for such adhesion, collectively referred to as "cell adhesion molecules" (CAM). One class of CAM is termed "cadherin". "Cadherin" is the term applied to a family of glycoproteins found in most kinds of mammalian tissues and thought to be responsible for  $\text{Ca}^{2+}$ -dependent cell-cell adhesion, (Takeichi, M., Development, 102:639 (1988)). Three subclasses of cadherin have been identified, namely, E-cadherin (from epithelial tissues), P-cadherin (from placental tissues), and N-cadherin (from neural tissues) (Yoshida-Noro, C., et al., Dev. Biol., 101:19 (1984); Nose, A., et al., J. Cell Biol., 103:2649 (1986); Hatta, K., et al., Nature, 320:447 (1986)).

The different cadherins exhibit distinct tissue distribution patterns (Takeichi, U., (1988) above). E-cadherin, which was found to be distributed exclusively in epithelial cells of various tissues (Hatta, K., et al., Proc. Nat'l. Acad. Sci. (USA), 82:2789 (1985); Takeichi, 1988, above), appears to be

identical to uvomorulin (Hyafil, F., et al., Cell, 21:927 (1986)), chicken liver-cell adhesion molecule (L-CAM, Gallin, W.J., et al., Proc. Nat. Acad. Sci. (USA), 80:1038 (1983)), and cell-CAM 120/80 (Damsky, C.H., et al., Cell, 34:455 (1983)) in terms of biochemical properties (Cunningham, B.A., et al., Proc. Nat. Acad. Sci. (USA), 81:5787 (1984)) and tissue distributions (Thiery, J.-P., et al., Dev. Biol., 102:61 (1984)).

10 N-cadherin, which is expressed in various neural tissues including astrocytes (Hatta, K., et al., Devel. Biol., 120:215 (1987); Matsunaga, M., et al., Nature, 334:62 (1988); Tomaselli, K.J., Neuron, 1:33 (1988)), shows 92% amino acid sequence homology between  
15 mammalian and avian homologs, shows from 40 to 50% similarity to epithelial E-cadherin and to placental P-cadherin of the same species, but was immunologically not cross-reactive with other cadherins within the same animal (Miyatani, S., Science, 245:631 (1989)).

20 Placental P-cadherin has also been cloned, and the deduced amino acid sequence of this glycoprotein was found to exhibit about 58% homology with epithelial E-cadherin (Nose, A., et al., EMBO J., 12:3655 (1987)).

Subsequent to the September 27, 1989 filing of the  
25 parent application, Heimark, et al. (Heimark, R.L., et al., J. Cell Biol., 110:1745 (1990) reported on the identification of a  $\text{Ca}^{2+}$ -dependent cell-cell adhesion molecule in aortic endothelial cells.

Although each of the aforelisted cadherins  
30 displays unique immunological and tissue distribution specifications, all have features in common: (1) a requirement for  $\text{Ca}^{2+}$  for cell adhesion function; (2) protection by  $\text{Ca}^{2+}$  from proteolytic cleavage; (3) similar numbers of amino acids, i.e., from about 723 to  
35 about 822; (4) similar masses, i.e., about 124 kdal.



for the glycoprotein; (5) substantial interspecies (50%-60%) overall sequence homology with interspecies homologies increasing to about 56% to 99% in the cytoplasmic region of the protein, suggesting that they constitute a gene family (Nose, A., 1987; Miysysni, D., et al., 1989); and (6) a common mechanism of action, namely, homophilic binding of cadherins on one cell to similar cadherins on the adjoining cell.

- CAMs independent of  $\text{Ca}^{2+}$  are also known, for example, the 125K glycoprotein of Urushihara et al. (Urushihara, H., et al., Cell, 20:363 (1980)); N-CAM (Rutishauser, U., Nature, Lond., 310:549 (1984)); Ng-CAM (Grunet, M. et al., Proc. Nat'l. Acad. Sci. (USA), 81:7989 (1984)); L1 (Rathjien, F.G. et al., \*\*\*\*\* J., 3:1 (1984)); G4 (Rathjien, F.G. et al., J. Cell Biol., 104:343 (1987)); and platelet glycoprotein PECAM-1 (CD 31) (Newman, P.J., Science, 247:1219 (1990)).  $\text{Ca}^{2+}$ -independent CAMs are known to exhibit certain properties of the  $\text{Ca}^{2+}$ -dependent CAMs. Thus, N-CAM and N-cadherin both promote retinal neurite outgrowth on astrocytes (Neugebauer, K.M., et al., J. Cell Biol., 107:1177 (1985)), and on Schwann cells (Bixby, J.L. et al., J. Cell Biol., 107:353 (1988)).

- Monoclonal antibodies raised against epithelial E-type cadherins such as uvomorulin are known to disrupt the adhesion of several cell types, including embryo cells, cultured teratocarcinoma cells, hepatocytes, and MDCK kidney epithelial cells (Ogou, S.-I., et al., J. Cell Biol., 97:944 (1983); Yoshida-Noro, et al., (1984), above; Shirayoshi, Y., et al., Cell Struct. Funct., 11:285 (1986); Gallin, et al., (1983), above; Vestweber, D., et al., EMBO J., 4:3393 (1985); Johnson, M.H., et al., J. Embrol. Exp. Morphol., 93:239 (1986); Gumbiner, B., et al., J. Cell Biol., 102:457 (1986)).

However, prior to the present discoveries disclosed in the parent applications cadherins had not been found in brain capillary or other endothelial cells (see, Takeichi, et al. (1988), above). Further,

5 the CAMs of microvascular endothelial cells had not yet been identified, nor had such molecules been localized specifically to brain capillary endothelial cells. Thus, until the present invention no means were known for transiently and reversibly disrupting tight  
10 junctions between microvascular endothelial cells, including those of the BBB, based upon an attack upon the CAM's of such cells that are responsible for tight junction formation and maintenance.

It has been hypothesized that the cadherins  
15 contain a common cell adhesion recognition (CAR) sequence. The CAR sequences of several cell and substratum adhesion molecules are known. Martin, G.R., et al., Ann. Rev. Cell Biol., 3:57 (1987) ; Ruoslahti, E., et al., Science, 238:491 (1987). In general, CAR  
20 sequences are composed of at least three amino acid residues. The most rigorously investigated CAR sequence is RGD which is found in laminin, fibronectin and other basement membrane components that are responsible for the binding of cells to the substratum.

25 Blaschuk, et al., in a paper to be published subsequent to the filing of the present application (Blaschuk, O., et al., J. Mol. Biol., in press, (1990)), disclose the presence of three potential cadherin CAR sequences in the first extracellular  
30 domains of liver CAM, E-, P-, and N-cadherin, namely, PPI, GAD and HAV. Blaschuk, et al. (Blaschuk, O., et al., Develop. Biol., 139:227 (1990)), also disclosed recently that synthetic peptides containing the HAV sequence inhibited two biological processes (compaction  
35 of 8-cell-stage mouse embryos and rate of neurite

outgrowth on astrocytes) that are known to be mediated by cadherins. Effective peptides in these assays were LRAHAVDVNG and AHAVSE; PPI-containing peptides were without effect. However, Blaschuk et al. provide no  
5 guidance for determining the regions flanking the HAV tripeptide that are critical for cell-cell adhesion. In the BBB disrupting peptides of the present invention detailed below, we have observed that the mere presence of the HAV sequence in a small cadherin-derived peptide  
10 is not the sine qua non for a composition effective to prevent cell-cell adhesion. Indeed, it should be emphasized that neither Blaschuk et al. nor any other publication known to the present inventors suggest that cadherin sequences containing HAV or SHAVS sequences  
15 would be effective in opening tight junctions and piercing blood brain barriers formed by E-cadherins in brain microvascular endothelial cells.

#### SUMMARY OF THE INVENTION

It has now been discovered that molecules  
20 homologous to, and immunologically related to, cadherin cell adhesion molecules are present on brain and non-brain microvascular endothelial cells, such that

junctions between such endothelial cells can be reversibly opened so as to permit passage of therapeutic drugs by the use of polypeptide and antibody compositions that compete with such cell  
5 adhesion molecules for binding to such cells.

It is therefore an object of this invention to provide the identity of microvascular endothelial cell adhesion molecules.

Another object of this invention is to provide DNA  
10 sequences of genes, and plasmids containing same, coding for the expression of all or a cell-binding portion of microvascular endothelial cell adhesion molecules.

Yet another object of this invention is to provide  
15 means to identify those sequences of cell adhesion molecules responsible for the tight binding of adjoining endothelial cells.

A further object is to provide therapeutic compositions comprising polypeptides derived from cell  
20 adhesion molecules that reversibly disrupt cell-cell adhesion.

Still another object of this invention is to provide therapeutic compositions comprising polyclonal or monoclonal antibodies or fragments thereof directed  
25 against endothelial cell adhesion molecules, or against polypeptides representing cell binding regions thereof, that reversibly disrupt endothelial cell-cell adhesion.

Yet another object of this invention is to provide therapeutic formulations comprising therapeutic drugs  
30 conjugated with blood-brain barrier-disrupting compositions of this invention, that are capable of entering the central nervous system following disruption of the blood-brain barrier.

These and other objects of this invention will  
35 become clear by reference to the following description

of the invention and to the appended claims.

#### DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to chicken N-cadherin.

Figure 2 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to mouse P-cadherin.

Figure 3 illustrates the cDNA sequence for the MDCK cell adhesion molecule homologous to mouse E-cadherin.

Figure 4 illustrates the restriction sites in the bovine endothelial cell N- (4-1 to 4-5) and P-cadherin (4-6 to 4-8) cDNA sequences and in the MDCK E-cadherin (4-9 to 4-14) cDNA sequence.

Figure 5 shows the staining of a mouse brain thin section by an antibody raised against a fusion protein derived from amino acids 9-96 of MDCK E-cadherin containing an HAV region.

Figure 6 is a repeat of the experiment of Fig. 5, except that the antibody was raised against the entire E-cadherin molecule.

Figure 7 illustrates the effects of an 18-mer HAV-containing polypeptide on the resistance of tight junction monolayers of MDCK epithelial cells.

Figure 8 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight junction monolayers MDCK epithelial cells.

Figure 9 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight-junction monolayers of brain microvascular endothelial cells.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that cell adhesion molecules with characteristics of cadherins are present on the surfaces of brain capillary endothelial cells and of microvascular endothelial cells of non-brain origins. The present invention is based on the discovery that a polypeptide composition comprising cell binding domains of endothelial cell adhesion molecules may compete against such molecules for binding to such cells, such that by this means the junctions between such cells could be reversibly opened, thereby permitting penetration by therapeutic agents. The present invention also discloses that polyclonal or monoclonal antibodies (or fragments thereof) raised against endothelial cell adhesion molecules or cell-binding domains thereof may also compete for endothelial cell surface binding sites, and, by this means, reversibly disrupt junctions between endothelial cells, thereby permitting entry into the central nervous system of therapeutic agents.

In order to obtain compositions useful for disrupting tight junctions between microvascular endothelial cells, the cell adhesion molecules responsible for such junctions were identified.

The endothelial cell cadherins disclosed herein exhibit one or more of several characteristics of E-, P- and N- cadherins, including: characteristics of a transmembrane integral protein, with cytoplasmic, hydrophobic plasma membrane, and extracellular regions; intraspecies DNA sequence homologies of greater than about 50% for the entire molecule; immunological cross-reactivity with antibodies raised against non-endothelial cell cadherins; and containing cell-binding domains. "Immunologically related to" means that these cadherin-like molecules cross-react with antibodies

raised against non-endothelial cell cadherins.

E-cadherin-like molecules were localized in brain by immunofluorescence. Cryostat sections of mouse brain were labeled with a rabbit antibody prepared  
5 against E-cadherin, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. There is clear labeling of a capillary in brain sections as shown by immunofluorescence microscopy. Endothelial cells in liver and kidney were  
10 not stained by this procedure.

cDNAs coding for the expression of bovine microvascular endothelial cell (BMEC) cadherins were cloned and sequenced as described below, and the partial sequence of N-cadherin and P-cadherin are  
15 disclosed herein in Figures 1 and 2, respectively. In addition, as MDCK dog kidney epithelial cells are known to employ E-cadherin to form high resistance tight junctions, and as the present invention discloses that brain capillary endothelial cell adhesion molecules  
20 include E-type cadherin, the DNA of this cadherin was also cloned; its complete DNA sequence is disclosed herein (Fig. 3).

N-, P- and E-cadherin-type clones described herein were deposited in the American Type Culture Collection  
25 on September 26, 1989, and were assigned the following accession numbers:

	<u>Clone Designation</u>	<u>Accession No.</u>
	N-cadherin-type clones	
	pUC19-bNCad 10A	40667
	pUC19-bNCad 39A	40669
5	P-cadherin-type clones	
	pUC18-bPCad 3B-10	40668
	pUC19-bPCad 9B	40670
	E-cadherin-type clones	
	pBluescript MDCKECad 45-30E	40671

10       The cloning of cadherins was accomplished by taking advantage of the fact that the cadherins characterized thus far are transmembrane glycoproteins, the cytoplasmic domains of which are highly conserved, that is, are highly homologous.

15       Two degenerate oligonucleotides flanking the 42-amino acid coding region in the cytoplasmic domain were selected to serve as primers for polymerase chain reaction (PCR) using either BMEC cDNA or MDCK cDNA as templates. The PCR reactions were carried out  
20 essentially according to Saiki, R. K. et al., Science, 239:487 (1988), which is incorporated herein by reference.

      The cloned PCR products from each cell type were sequenced essentially according to the method of  
25 Sanger, F. et al., Proc. Nat'l. Acad. Sci. (USA), 74:5463 (1977), which is incorporated herein by reference.

      It was discovered that BMEC cadherins are of two types - one homologous to chicken N-cadherin (neuronal  
30 type, see, e.g., Hatta, K., et al., J. Cell Biol., 106:873 (1988)) and the other homologous to mouse P-cadherin (placental type, see e.g., Nose, A., et al., (1987) above). It has also been found that there are two species of cadherins in MDCK cells - one homologous



to mouse E-cadherin (see, e.g., Nagafuchi, A., et al., Nature, 329:341 (1987)) and the other homologous to mouse P-cadherin (Nose, et al. (1987), above).

The PCR products were then used as probes to  
5 isolate the BMEC and MDCK cadherin cDNA clones as follows. A cDNA library was constructed essentially according to Gubler et al. (Gubler, U. et al., Gene, 25:263 (1983), which is incorporated herein by reference), using poly (A)<sup>+</sup>RNA isolated from either  
10 BMEC or MDCK cells. The cDNA was ligated via EcoRI adaptors into gt10 arms (BMEC) or ZAP<sup>R</sup> (from Stratagene, Inc., La Jolla, CA) vector arms (MDCK). cDNA libraries containing  $5 \times 10^5$  -  $1.5 \times 10^6$  independent cDNA clones were screened using  
15 radiolabeled PCR products (Benton, W.D. et al., Science, 196:180 (1987), which is incorporated herein by reference). Northern blot analysis (Maniatis, T. et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.,  
20 1982) may be used to determine whether each cDNA species cloned hybridizes to a single mRNA species, as well as the tissue distributions of each cDNA species.

cDNA clones for each cadherin were sequenced by the method of Sanger et al. (1977) above.

25 The partial restriction maps for each cDNA clone based on their sequences are shown in Fig. 4. Some of these restriction sites were confirmed by restriction enzyme digestions, including Hind III, Pst I, Kpn I, Bgl II for N-cadherin; Pvu II, Sac I and Pst I for  
30 P-cadherin; Pst I, Pvu II, BamH I, and Sac I for E-cadherin.

In order to test whether the cloned E-cadherin cDNA contains all the information necessary for cadherin function, full-length E-cadherin cDNA joined  
35 to a suitable promoter may be introduced into mouse

L-cells that have very little endogenous cadherin activity (Nagafuchi, et al. (1987), supra). To test for expression of E-cadherin in transfectants derived from the introduced cDNA, transfected L-cells may be  
5 tested for  $\text{Ca}^{2+}$ -dependent aggregating activity. The extent of this aggregating activity should be closely correlated with the amount of E-cadherin expressed (Takeichi, M. (1988), supra). This same technique may be used for testing cDNAs encoding bovine endothelial  
10 N- and P-cadherins, according to the method of Hatta, et al. (Hatta, K., et al. (1988), supra).

In order to identify cell binding domains in, for example, MDCK E-type cadherin, L-cells may be first transfected as above with a cDNA of a size sufficient  
15 to cause  $\text{Ca}^{2+}$ -mediated aggregation of transfectants. A series of deletion mutants comprising truncated cDNA species missing different regions of the extracellular domain may be prepared by restriction enzyme digestion and proper end filling or exonuclease digestion to make  
20 the deletions in the proper coding frames. These deletion mutants can then be tested for their ability to express in L-cells a protein causing  $\text{Ca}^{2+}$ -dependent aggregation. By correlating a loss of aggregation with deletion of particular fragments, the regions important  
25 for cell binding may be determined. A variety of polypeptides corresponding to binding regions of cadherins, as deduced from the nucleotide sequences of deleted cDNA, may be synthesized chemically using an automated peptide synthesizer such as that of Applied  
30 Biosystems, Inc., Foster City, CA, or expressed by recombinant DNA methods. Effective polypeptides may be of varying lengths, depending upon the natures of junctions being disrupted and the cell adhesion molecule present.

Nucleotide, and corresponding amino acid, sequences of cadherins may be analyzed to detect homologous regions. Applying this technique to bovine endothelial cell N- and P-cadherins and to epithelial cell E-cadherin, we have determined that, in the amino acid 80 region of each of these cadherins, there is conserved a triplet HAV (His-Ala-Val) region. We have deduced that this HAV region may be a common cell adhesions recognition (CAR) sequence.

We have chemically synthesized the following polypeptides, each of which containing the HAV sequence:

6-mer(78-83)	NH <sub>2</sub> -SHAVSS-CONH <sub>2</sub>
11-mer(76-86)	NH <sub>2</sub> -LYSHAVSSNGN-CONH <sub>2</sub>
17-mer(74-90)	NH <sub>2</sub> -YILYSHAVSSNGNAVED-CONH <sub>2</sub>
18 mer(69-86)	NH <sub>2</sub> -EQIAKYILYSHAVSSNGN-CONH <sub>2</sub>
20-mer(71-90)	NH <sub>2</sub> -IAKYILYSHAVSSNGNAVED-CONH <sub>2</sub>

and have tested each for efficacy in opening brain endothelial cell tight junctions in the BBB model disclosed in copending United States application Serial No. 07/413,274, and also on kidney epithelial cell tight junctions..

Polyclonal antibodies raised in rabbits and monoclonal antibodies derived from hybridomas may be generated against each of the chemically-synthesized polypeptides by standard methods. (Harlow, E., et al., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Goding, J.W., "Monoclonal Antibodies: Principles and Practice", Academic Press, N.Y. 1986). In addition, recombinant antibodies may be prepared. Fragments of antibodies, e.g., Fc, Fab, F(ab)', may be prepared by standard methods.

We have cloned and sequenced fusion proteins derived from amino acids 9-96 of MDCK E-cadherin

containing the HAV region. A polyclonal antibody prepared against this fusion protein stained rat (Fig.55) mouse brain sections as well as did an antibody raised against the entire E-cadherin (Fig. 6).

- 5 A polyclonal antibody raised against a fusion protein derived from amino acids 9-37 failed to stain brain sections. These results indicate that the key cell-binding domain of E-cadherin lies in the region of amino acids 37-96.

- 10 The ability of CAM-derived polypeptides containing cell-binding domains, and the corresponding polyclonal and monoclonal antibodies, of the invention to disrupt tight junctions may be tested in in vitro and in vivo models of high resistance tight junctions and in animal  
15 models. Monolayers of MDCK dog kidney epithelial cells, that are known to contain high resistance tight junctions (Gumbiner, B., J. Cell Biol., 102:457 (1986)), can be used to test for the ability of the polypeptides and corresponding antibodies of the  
20 present invention to disrupt such tight junctions.

- Polyclonal antibodies prepared as described above may also be used in conjunction with Western blotting (Old, R.W., et al., Principles of Gene Manipulation, 3d ed., Blackwell, Oxford, 1985, p. 10) and a variety of  
25 tissue extracts in order to identify cell adhesion glycoproteins in such extracts.

- Another embodiment of the present invention is in drug delivery systems. Conjugates between therapeutic drugs and agents that affect cell adhesion molecule  
30 function in brain capillary endothelial cells may be used to deliver therapeutic drugs to the CNS. For example, a polypeptide derived from a cell adhesion molecule that contains within its amino acid sequence a cell-binding domain, or antibodies thereto, may be  
35 conjugated in biologically-active form to a therapeutic

modality. Such conjugates may have the dual effect of opening the BBB and delivering the therapeutic agent to the brain side of the BBB. Delivery of therapeutic drugs to the CNS, either alone or conjugated to agents that disrupt cell-cell adhesion, may be accomplished by administering such drugs to a subject either simultaneously with or subsequent to the administration of the agents of this invention that disrupt the tight junctions of the BBB. Examples of therapeutic modalities that may be delivered to the brain by the cell adhesion disruption compositions of this invention include Nerve Growth Factor, anti-Parkinsonian drugs, and brain enzymes known to be missing in sphingolipidoses, e.g., Tay-Sachs disease. Means of chemically conjugating protein or polypeptide carriers to therapeutic agents such that the biological integrity of the therapeutic agent is not compromised and such that the therapeutic agent is readily cleaved from the carrier by enzymes present on or within endothelial cells (e.g., amidases, esterases, disulfide-cleaving enzymes), are well known in the art. It is also apparent that these therapeutic conjugates may be delivered to endothelial cells in encapsulated form (e.g., in liposomes) or as microsuspensions stabilized by pharmacological excipients.

It is known (Jain, R.K., J. Natn'l Cancer Inst., 81:570 (1989)) that many solid tumors develop internal barriers, including high pressure zones and collapsed blood vessels, that make it difficult for blood-borne chemotherapeutic agents to reach the tumor's inner core. The barrier problem is particularly troublesome with therapeutic products drawn from the human immune system, such as monoclonal antibodies conjugated with chemotherapeutic agents, interleukin-2, interferon and activated killer T-lymphocytes, because of their large

size. Thus, in another embodiment of this invention, compositions that disrupt the junctions between endothelial cells, particularly the relatively small peptides that contain one or more cell-binding regions of cell adhesion macromolecules, may be used to enhance drug delivery to tumors with depressed blood flow.

It has been theorized that cancer cells metastasize by secreting soluble cadherins variously to open tight junctions in cells that block their movement and to prevent their being bound to such cells. We consider it likely that antibodies raised against these cadherins, which are derived from extracellular domains of the cadherins disclosed in this invention, may provide a therapeutic modality that inhibits or prevents cancer cell metastases.

In another embodiment, the compositions of this invention may also be used to provide penetration for chemotherapeutic agents of other well-known blood-tissue barriers, such as blood-testis barriers and blood-retina barriers. The latter barrier is known to prevent the efficient transport of, for example, administered antibiotics to the retina from the general circulation. The cell adhesion disrupting compositions of this invention may, thus, be used in conjunction with the administration of antibiotics to treat retinal infections.

The following examples are illustrative of several embodiments of this invention, and should not be construed in any way as limiting the invention as recited in the claims.

#### EXAMPLE 1

##### EFFECTS OF HAV-CONTAINING POLYPEPTIDES ON TIGHT JUNCTIONS OF MDCK EPITHELIAL AND BOVINE ENDOTHELIAL CELLS

The BBB model of copending U.S. Serial No. 07/413,332 was used to examine the effects of polypeptides containing the HAV region on the tight junctions of monolayers of MDCK epithelial cells and  
5 bovine capillary endothelial cells as determined by resistance measurements across the monolayers.

The polypeptide was added to the cells either from the apical side (top) or basolateral side (bottom), as shown in the following sketch.

10

APICALEPITHELIAL CELLS  
Gut SideENDOTHELIAL CELLS  
Blood Side

Blood Side

Brain Side

BASOLATERAL

15 Figure 7 illustrates the effects of various concentrations of the aforementioned 18-mer polypeptide on resistance of MDCK epithelial cells. At the lowest concentration tested, 0.5 mg/ml, resistance was  
20 markedly decreased. The polypeptide was more effective when added from the basolateral side, but at high concentrations was quite effective even when added from the apical side. These data indicate that the 18-mer is effective in making tight junctions permeable. The  
25 20-mer was similarly effective, and a 17-mer less effective.

Figure 8 illustrates the effects of the aforementioned 11-mer and 18-mer on MDCK cell resistance when added from either the apical or  
30 basolateral side of the monolayers. The concentration of polypeptide was about 1 mg/ml. The 11-mer (as well

as the 6-mer data not shown) was virtually without effect. With the 18-mer, resistance was almost totally abolished by about 6 hours, indicating disruption of tight junctions. That the effect of the 18-mer is

5 reversible is indicated by the "wash-out" experiment. When the 18-mer was washed out of the MDCK cells at 6 hours, resistance recovered to a substantial extent over the next 21 hours. This recovery was particularly pronounced when the 18-mer had originally been added  
10 from the basolateral side of the monolayers. The 20-mer produced results similar to those of the 18-mer, and the 17-mer was effective, but somewhat less so.

Figure 9 illustrates the effect of 1 mg/ml of the 11-mer and 18-mer on high resistance monolayer cultures  
15 of brain endothelial cells (see copending United States Serial No. 07/413,332 for method of preparation). As with MDCK cells, the 11-mer (and the 6-mer) failed to reduce resistance values over a 48-hour period of observation. In contrast, the 18-mer (as well as the  
20 20-mer) decreased resistance values markedly when added from either the basolateral or apical side, but the effect of the polypeptide was more rapid and more pronounced when it was added from the basolateral side; the 17-mer was less effective.

25 The conclusion of these experiments is that a particular set of peptides (but not all peptides) centered around the HAV region of E-cadherin are effective in opening tight junctions of brain endothelial cell blood-brain barriers, and also of  
30 epithelial cells that form such junctions ("gut barrier"). Both the length and composition of the amino acid region flanking the HAV triplet thus appear to play a role in the efficacy of such compositions.

While the aforementioned embodiments represent the  
35 preferred embodiments of the invention, those skilled



in the art may, without undue experimentation, devise other executions of the compositions and methods of use of this invention without departing from the concept and spirit inherent therein.

What is claimed is:

1. A composition for opening tight junctions between microvascular endothelial cells of a subject, whereby means are provided for a drug to cross the permeability barrier imposed by such junctions, comprising an agent capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted.
2. A composition of claim 1, wherein said cell adhesion molecule exhibits at least about 50% sequence homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.
3. A composition of claim 1, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.
4. A composition of claim 1, wherein the microvascular endothelial cells are brain capillary endothelial cells.
5. A composition of claim 2, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
6. A composition of claim 3, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
7. A composition of claim 5, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.
8. A composition of claim 7, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

9. A composition of claim 8, wherein said cell-binding domain contains an HAV amino acid sequence.

10. A composition of claim 9, wherein said amino acid sequence is



11. A composition of claim 9, wherein said amino acid sequence is



12. A composition of claim 9, wherein said amino acid sequence is



13. A composition of claim 9, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

14. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

15. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

16. A composition of claim 15, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

17. A composition of claim 16, wherein said cell-binding domain contains an HAV amino acid sequence.

25

18. A composition of claim 17, wherein said amino acid sequence is



19. A composition of claim 17, wherein said amino acid sequence is



20. A composition of claim 17, wherein said amino acid sequence is



21. A composition of claim 17, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

22. A composition of claim 5 or 6 in a pharmaceutically-acceptable vehicle.

23. A method for opening tight junctions between microvascular endothelial cells of a subject, comprising the step of administering to the subject an agent, in an effective amount and in a  
5 pharmaceutically-acceptable vehicle, capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted and  
10 whereby means are provided for a drug to cross permeability barriers imposed by such tight junctions.

24. A method of claim 23, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

25. A method of claim 23, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

26. A method of claim 23, wherein the microvascular endothelial cells are brain capillary endothelial cells.

27. A method of anyone of claims 23-25, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

28. A method of claim 27, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

29. A method of claim 28, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

30. A method of claim 29, wherein said cell-binding domain contains an HAV amino acid sequence.

31. A method of claim 30 wherein said amino acid sequence is



32. A method of claim 30, wherein said amino acid sequence is



33. A method of claim 30, wherein said amino acid sequence is



34. A method of claim 30, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

35. A method of claim 27, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

36. A method of claim 28, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said fragment of said cell adhesion molecule.

37. A method of claim 36, wherein said cell adhesion fragment includes within its amino acid sequence a cell-binding domain.

38. A method of claim 37 wherein said cell-binding domain contains an HAV amino acid sequence.

39. A method of claim 38, wherein said amino acid sequence is



40. A method of claim 38, wherein said amino acid sequence is



41. A method of claim 38, wherein said amino acid sequence is



42. A method of claim 38, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

43. A drug delivery composition comprising a conjugate between a therapeutic drug and an agent capable of reacting with at least one type of a cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is

disrupted by said agent, whereby means are provided for said drug to cross permeability barriers imposed by such tight junctions, in a pharmaceutically-acceptable  
10 vehicle.

44. A drug delivery composition of claim 43, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

45. A drug delivery composition of claim 43, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

46. A drug delivery composition of claim 43, wherein the microvascular endothelial cells are brain capillary endothelial cells.

47. A drug delivery composition of any one of claims 43-45, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

48. A drug delivery composition of claim 47, wherein said agent comprises a fragment of said cell adhesion molecule.

49. A drug delivery composition of claim 48, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

50. A drug delivery composition of claim 49, wherein said cell-binding domain contains an HAV amino acid sequence.

51. A drug delivery composition of claim 50, wherein said amino acid sequence is



52. A drug delivery composition of claim 50, wherein said amino acid sequence is



53. A drug delivery composition of claim 50, wherein said amino acid sequence is



54. A drug delivery composition of claim 50, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

55. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

56. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

57. A drug delivery composition of claim 56, wherein said cell adhesion molecule fragment contains within its amino acid sequence a cell-binding domain.

58. A drug delivery composition of claim 56, wherein said cell-binding domain encompasses an HAV amino acid sequence.

59. A drug delivery composition of claim 58, wherein said amino acid sequence is



60. A drug delivery composition of claim 58, wherein said amino acid sequence is



NH<sub>2</sub>-EQIAKYILYSHAVSSNGN-COHN<sub>2</sub> .

61. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH<sub>2</sub>-IAKYILYSHAVSSNGNAVED-CONH<sub>2</sub> .

62. A drug delivery composition of claim 58, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

63. A drug delivery composition of claim 43, wherein said conjugate comprises a physiologically-cleavable covalent bond.

64. A drug delivery composition of claim 43, wherein said conjugate is encapsulated within a physiologically-compatible particle.

65. A drug delivery composition of claim 64, wherein said particle comprises a liposome.

## FIG. 1a.

Partial cDNA sequence for the bovine endothelial N-cadherin

GAATTCGAAC CCCTTCGTTT CATTATGCAA GACTGGATTT CCTGAAGATG TGTACAGTGC	60
AGTCTTGTC CGGGATGTGC TGGAAGGACA GCCCCTTCTC AATGTGAAGT TTAGCAACTG	120
CAATGGGAAA AGAAAAGTAC AGTATGAGAG CAGCGAGCCA GCAGATTTTA AGTGGATGA	180
AGATGGCATG GTGTATGCCG TGAGAAAGCTT CCCCCTCTCA TCTGAACACT CGAAGTTCCT	240
GATATACGCT CAAGACAAAG AGACTCAGGA AAAGTGCAA GTAGCAGTAA AACTGAGCCT	300
CAAAACCAGCC CTACCTGAGG ATTCAGTGAA GGAATCACGA GAAATAGAAG AAATAGTGTT	360
TCCAAGACAA GTGACTAAGC ACAATGGCTA CCTGCAGAG CAGAAGAGAG ACTGGGTAT	420
CCCTCCCATC AACTTGCCAG AAAACTCCAG AGGGCCCTTT CCTCAAGAGC TCGTCAGGAT	480
CAGATCCGAT AGAGATAAAA ACCTTCTCT GCGGTACAGC GTAACTGGGC CAGGAGCTGA	540
CCAGCCTCCA ACTGGTATCT TCATTATCAA CCCCATCTCA GGTCAAGTGT CAGTAACCAA	600
GCCTCTGGAT CGTGAGCTGA TAGCCCCGGT TCATTGAGG GCACATGCAG TGGATATTAA	660
TGGAACCAA GTGGAGAACC CCATCGACAT TGTCAATCAAC GTTATTGACA TGAATGATAA	720
CAGACCTGAG TTCTTACACC AGGTTTGAA TGGACAGTT CCTGAGGGAT CAAAGCCGGG	780
AACATATGTG ATGACGGTCA CTGCGATTGA TGCTGACGAT CCAATGCCC TCAATGGGAT	840

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FIG. 1b.

GTTGAGGTAC AGAATCCTGT CCCAGGCGCC AAGCACCCCT TCGCCCAACA TGTTTACAAT	900
CAACAATGAG ACTGGGACA TTATCACGGT GGCAGCTGGA CTTGACAGAG AAAAAGTACA	960
ACAGTATACG TTAATAATTC AAGCTACAGA CATGGAAGGC AATCCCACAT ATGGCCTTTC	1020
CAACACAGCC ACGGCTGTCA TCACGGTGAC AGATGTCAAC GACAATCCTC CGGAGTTTAC	1080
TGCCATGACG TTCTATGGTG AAGTCCCTGA AACACGGTA GATGTCATCG TCGCTAATCT	1140
AACAGTGACA GATAAGGATC AGCCCCACAC ACCGGCCTGG AACGCCATCT ACAGAATCAG	1200
CGGTGGAGAC CCCGCCGGCC GCTTTGCCAT TCAAACTGAC CCCAACAGCA ACGACGGTTT	1260
AGTCACCGTA GTAAAACCAA TCGACTTTGA AACAAATAGG ATGTATGTCC TTA CTGTGCGC	1320
TGCAGAAAAT CAAGTGCCAT TAGCCAAGGG TATTCAGCAT CCACCTCAGT CAACTGCGAC	1380
TGTGTCTGTC ACAGTTATCG ATGTGAATGA AAATCCTTAT TTTGCCCCAA ATCCAAAGAT	1440
CATTCCGCCAA GAAGAAGGCC TTCACGCCGG TACCGTGTTA ACAACGTTTA CTGCTCAGGA	1500
CCCAGATCGA TATATGCAGC AAAATATCAG ATACACCAA TTATCCGATC CTGCAAACTG	1560
GCTAAAATA GACTCTGTGA ATGGGCAGAT AACTACCATT GCTGTTTGG ACAGAGAATC	1620
ACCGAATGTG AAAGCCAATA TATACAATGC TACTTTCCTT GCTTCTGACA ATGGAATCCC	1680
TCCTATGAGT GGAACGGGAA CACTGCAGAT CTATTTACTT GATATTAATG ACAATGCCCC	1740

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FIG. 1c.

TCAAGTGTTA CCTCAAGAGG CAGAGATTG TGAAACTCCG GACCCCAATT CAATTAACAT 1800  
CACAGCACTT GATTATGACA TTGATCCAAA TGCTGGACCA TTTGCTTTTG ATCTTCCTTT 1860  
GTCTCCAGTG ACTATTAAAG GAAATTGGAC CATCACTCGG CTTAATGGTG ATTTTGCTCA 1920  
GCTTAACTTA AAGATAAAAT TTCTTGAGGC CGGATCTAC GAAGTTCCAA TCATAATCAC 1980  
AGATTGCGGT AATCCTCCCA AATCGAATAT CTCCATCCTT CCGGTGAAGG TTTGCCAGTG 2040  
TGATTCCAAC GGGGACTGCA CAGATGTGGA TCGAATTGTG GGAGCAGGCG TGGGCACCGG 2100  
CGCCATCATC GCCATCCTGC TTGTCATCAT CATCCTGCTC ATTCTCGTTC TGATGTTTCT 2160  
GGTATGGATG AAACGCCGGG ATAAAGAACG CCAGGCCAAA CAACTTTTAA TTGATCCAGA 2220  
AGATGATGTA AGAGATAATA TTTTAAATA TGATGAAGAA GGTGGAGGAG AAGAAGACCA 2280  
GGACTACGAT TTGAGCCAGC TCCAGCAGCC TGATACGGTA GAGCCAGATG CCATCAAGCC 2340  
AGTTGGAATC CGACGGTTGG ATGAGAGGCC CATCCATGCG GAGCCCCAGT ACCCGGTTG 2400  
ATCTGCAGCC CCACACCCAG GGGACATCGG GGACTTCATT AATGAGGGCC TTAAAGCTGC 2460  
TGACAACGAT CCCACCGCTC CGCCCTACGA CTCCCTCTTA GTCTTTGACT ATGAAGGCAG 2520  
TGGCTCCACG GCCGGGTCCT TGAGCTCCCT TAATTCCCTC AGTAGTGGAG GTGAGCAGGA 2580  
CTATGACTAT CTGAACGACT GGGGGCCCCG CTTCAAGAAA CTCGCTGACA TGTACGCTGG 2640

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FIG. 1d.

AGGTGATGAC TGAACCTCAG GGTGAACCTTG GTTTTGGAC AAGTACAAAC AATTGCAACT 2700  
GATATTCCCA AAAAGCATTC AGAAGCTAGG CTTTAACTTT GTAGTCTACT AGCACAGTGC 2760  
TTGCTGGAGG CTTTGGCAGA GGCTGCAAC CAATTGGGC TCAGAGGGAA TATCGGTGAT 2820  
CCAATACTGT TTGGAAAACA CTGAGCTCAG TTACACTTGA ATTTTACAGT ACAGAAGCAC 2880  
TGGGATTTTA TGTGCCTTTT TGTACCCTTTT TCAGATTGGA ATTAGTTTTA TGTTTAAAGC 2940  
TTTAATGGTA CTGATTCTG AATGATAAG TAAAAGACAA AATATTTTGT GGTGGGAGCA 3000  
GTAAGTTAAA CCATGATATG CTTGACACAG CTTTGTGTAC ATCGCATTTG CTTTATTATA 3060  
AAATATGGAA TTAACACAGC AAACCAACCA CTCATGGAGC AATTTTATTA CCTTGGGGGC 3120  
TGAGACCATG AGATTGGAAA ATGTACATTA TTTCTAGTTT TAGACTTTAG TTTCTTGTTT 3180  
TGTTTTTTTT TTCCACTAAA ATCTTAAAAC TTACGCAGCT GGTGCAAAAT AAAGGGAGTT 3240  
TTCATATCAC CAATTGTAG CAAAATTGAA TTTTTCATA AACTAGAATG TTAGACACAT 3300  
TTTGGTCTTA ATCCATGTAC ACTTTTTTAT TTACTGTATT TTTTCCACTT CACTGTAAAA 3360  
ATGGTATGTG TACATAATGT TTTATTGGCA TAGTCTATGG AGAAGTGCAG AAACCTCAGA 3420  
ACATGTGTAT GTATTATTG GACTATGGAT TCAGGTTTTT TGCATGTTTA TATCTTTCGT 3480  
TATGGATAA GTATTACAA AACAAAGTGA CATTGATTC AATTGTTGAG CTGTAGTTAG 3540  
AATACTCAAT TTTTAATTTT TTAATTTTTT TTATTTTTTA TTTTCTCTTT TTGTTTGGG 3600

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AGGAGAGAAA GTTCTTAGCA CAAATGTTTT ACATAATTG TACCAAAAAA AACAAAAA 3660  
AAAGGAAAGA CAAGAAATGA AAGGGGTGAC CTGACACTGG TGGTACTACT GCAGTGTGTG 3720  
TTTTTAAAAA AAAATGAAAA AAAAAAAGCT TTATAAACTGG AGAGACTTCT GACAAACAGCT 3780  
TTGCCTCTGT ATTGTGTACC AGAATATAAA TGATACACCT CTGACCCCAG CGTTCTGAAT 3840  
AAAATGCTAA TTTTGAAAAA AAAAAAAA AAAA 3875

FIG. 1e.

FIG. 2a.

partial cDNA sequence for the bovine endothelial P-cadherin

GAATTGGAAC CCTTCGCTG AGAACACAGT GAGCCACGAG GTGCAGAGGC TGACAGTGAC 60  
TGATCTGGAC GCCCCTAACT CACCAGCATG GCGTGCCACC TACCGCATCG TGGGAGGTGA 120  
CAACGGGGAC CATTTTACCA TCACTACTGA CCCCAGAGAC AACCAAGGTA TCCTGACCAC 180  
CCAGAAGGGC TTGGATTTTG AGGCCAAAAC CCAGCACACC CTGTACGTG AAGTGATCAA 240  
CGAGGTCCC TTTGTGGTGA AACTCCCGAC CTCCACAGCC ACCGTAGTGG TCCTCGTGGA 300  
GGATGTGAAT GAGCCACCCEG TGTTTGTCCC CCCGTCCAAA GTCATCGAAA TCCAGGAGGG 360  
CATCTCCACT GGGGAGCCTA TTTGTGCCTA CACTGCACGG GACCCAGACA AGGGAGTCA 420

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FIG. 2b.

GAAGATCAGT TACCACATCC TGAGAGACCC AGCAGGGTGG CTAGCGATGG ACCCAGACAG 480  
TGGACAAGTC ACTGCCGCAG GGGTCTTGGA CCGTGAGGAT GAGCAGTTTG TGAGAAACAA 540  
CATCTACGAA GTCATGTGCT TGGCCACAGA TGATGGGAGC CCTCCCACCA CTGGCACAGG 600  
GACCCTCCTG CTAACACTGA TGGACATCAA TGACCACGGT CCGGTCCCCG AGCCCCGTCA 660  
GATCACCATC TGCAACCAA GCCCTGTGCC CCAGGTGCTA AACATCACAG ACAAGGACTT 720  
GTCCCCCCAC ACTGCCCCCTT TCCAGGCCCA ACTCACACAT GACTCGGACG TCTATTGGAC 780  
AGCAGAAGTC AACGAGAAAG GAGACGCAGT AGCCTTGTCC CTGAAGAAGT TCCTAAAGCA 840  
AGGCGAATAC GATGTGCACC TTTCCCTGTC CGACCACGGC AACAAAGAAC AGCTGACAGT 900  
GATCAGAGCC ACCGTGTGTG ACTGCCACGG CAACATGGTG ACCTGCCGGG ACCCCTGGAC 960  
GTGGGGTTTC CTCCTCCCCA TCCTGGGTGC TGCCCTGGCT CTGCTGCTCC TTCTGCTGGT 1020  
GCTCCTATTG TTGGTGAGAA AGAAACGGAA GATCAAGGAA CCCCTTCTCC TCCCAGAAGA 1080  
TGATAACCCGT GACAACGTCT TCTACTACGG CGAAGAGGGG GGTGGCGAGG AGGACCAGGA 1140  
CTATGACATC ACCCAGCTCC ACCGGGTCT GGAGGCCCGG CCTGAGGTGG TTCTCCGCAA 1200  
CGATGTGGCA CCATCCTTCA TCCCCACACC CATGTACCGT CCTCGGCCAG CCAACCCAGA 1260  
TGAAATCGGC AACTTCATCA TTGAGAACCT GAAGGCAGCC AACACAGACC CCACGGCCCC 1320

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GCCCTACGAC TCCCTGTTGG TGTTCGACTA TGAGGGCAGT GGCTCCGATG CCGCCTCTCT 1380  
GAGCTCGCTC ACCTCCTCAA CCTCTGACCA GGACCAAGAC TACAACATATC TGAATGAGTG 1440  
GGGAGCCGC TTCAAGAAGC TGGCGGACAT GTACGGCGGG GGCCAGGACG ACTAGGACTC 1500  
CCTAAACGCC GGGCTGCAGC AGCGTCTCCA AGGGGTCACT ATCCCCACGT TGGCCAAAGGA 1560  
CTTTGCAGCT TGTGAGAAT TGGCCTTAGC AACTTGGAGG GAAGAGGCCT CGAAACTGAC 1620  
CTCAAAGGGG CAGGTCTCTA TGCCTTTTCAG AACGGAGGAA CGTGGGCAGT TTGATTTCAA 1680  
CAGTGAGCAC CTCTTAGCCT AAGCCAGGGC TGCTCAATT CTGGGAGTCT CCTCGCTACC 1740  
ATAAAATGCT CAGCGCTGGG TCCTGGGTTT TGA CTGACTC TGA CTTTCCC ATGATGGCTT 1800  
TTGCTCTGGA ATGGACCCCTT CTCCCTTAGTA ACAGGCCCTCT TACCACAATC TTCGTTTTTT 1860  
TTTTTTTAAAT GCTGTTTTCA AAAAGTGAGA GGCAGGTCCT CAACCAACCC CTGGAGCGCT 1920  
CCAGAAAGCC AGGCGTGCCC TCATGCATTT CTCTGTGGTC TCTTGGCCCC CAGACCTCCT 1980  
GTTTGATTGG ATAACGTCAT TTTTATACTG AGCACGTCTA AGTGGTCCTT TATTTTTTAT 2040  
TTTCCCTATC GAGTGCTGTA GATGAAGAGT GATGACAATC CTGTAAATGT ACTAGAACTT 2100  
TTTTTATTAA GGAACTTTTT CCCAAAAAAA AAAAAAAA AAAAAA 2156

FIG. 2c.

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FIG. 3a.

cDNA sequence for MDCK E-cadherin

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CGGGCACCCTG TGATTGCGCG AAGTCCTGCC GCCTCGCGCC GCCTCGCGCC CGGCTCTCGA    60
CCCCCGCCCG CCATGGGCCC TCGGTACGGC GCGGCCCCCG CGCTCCTGCT CCCGCTGCTG    120
CTGCTGCTGC AGGTCTCATC GGGGCTCTGC CAAGAGCCGG AGCCCTGCCG CCCTGGCTTT    180
GGCGCTGACA GCTACACGTT CACCGTGCCC CGGCGACACT TGGAGAGAGG CCGTGTCCCTG    240
GGCAGGGTGA GTTTTGAAGG ATGCACCGGT CTACCTAGGA CAGCCTATGT TTCTGATGAC    300
ACCCGATTCA AAGTGGGCAC AGATGGTGTG ATTACAGTCA AGCGGCCCTCT ACAACTTCAT    360
AAACCAGAGA TAAGTTTCTT TGTCCATGCC TGGGACTCCA GCCGCAGGAA GCTCTCCACC    420
AGAGTTAGGC TGAAGGCAGC GACGCACCAC CACCACCACC ATCATGATGC TCCCTCTAAA    480
ACCCAGACAG AGGTGCTCAC ATTTCCCAGT TCCCAGCATG GACTCAGAAG ACAGAAGAGA    540
GACTGGGTTA TCCCTCCTAT CAGCTGCCCG GAAAACGAGA AAGGCCCATT TCCTAAAAAC    600
CTGGTTCAGA TCAAGTCTAA CAGGGACAAA GAAATCAAGG TTTTCTACAG CATCACTGGC    660
CAAGGAGCTG ACGCACCTCC TGTGGTGTG TTTATATATG AAAGAGAAAC AGGATGGCTG    720
AAGGTGACTG AGCCTCTGGA TAGAGAACAA ATTGCTAAGT ACATTCTCTA CTCTCATGCC    780
GTATCTTCTA ATGGGAATGC GGTGAAGAC CCAATGGAGA TCGTGATCAC GGTGACAGAT    840
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FIG. 3b.

CAGAAATGACA	ACAAGCCCGA	GTTACACCCAG	GCAGTCTTCC	AAGGATCTGT	CACGGAAGGT	900
GCCCTTCCAG	GCACCTCTGT	GATGCAGGTG	ACAGCCACAG	ATGCCGATGA	TGATGTGAAT	960
ACCTACAACG	CTGCCATCGC	TTACAGCATC	CTCACACAAG	ACCCCCTCCT	GCCTAGCAGC	1020
ATGATGTCA	CTATCAACAA	GGACACAGGA	GTCATCAGCG	TGCTCACCAC	TGGGCTGGAC	1080
CGAGAGGTG	TCCCCATGTA	CACCTTGGTG	GTTCAGGGTG	CTGACCTGCA	AGGCGAAGGC	1140
TTAACTACAA	CTGCAACAGC	TGTGATCACA	GTCAC TGACA	TCAATGATAA	CCCCCCCATC	1200
TTCAACCCAA	CCACGTACCA	GGGACGGGTG	CCTGAGAACA	AGGCTAACGT	CGAAATCGCT	1260
GTA CTCAAAG	TGACGGGATGC	TGATGTCCCC	GATACCCCGG	CCTGGAGGGC	TGTGTACACC	1320
ATATTGAACA	ATAACAATGA	TCAATTGTGT	GTCACCACAG	ACCCAGTAAC	TAACGACGGC	1380
ATTTTGAAAA	CAACTAAGGG	CTTGGATTTT	GAGGACAAGC	AGCAGTATGT	CTTGTACGTG	1440
ACTGTGGTGA	ACGTGACCCC	GTTTGAGGTC	ATCCTCTCCA	CCTCCACAGC	CACGTGCACT	1500
GTGGACGTGG	AAGATGTGAA	TGAAGCCCCC	ATCTTCATCC	CTTGCCCAA	GGTAGTGTC	1560
ATCCCTGAAG	ACTTTGGTGT	GGGCCAGGAA	ATCACATCCT	ACACGCCCGA	GGATCCAGAT	1620
ACATATATGG	AACAGAGGAT	AACGTATCGG	ATTTGAGGGG	ATGCTGCCGG	TGCGCTGGAG	1680
GTTAATCCAG	AATCTGGTGC	CATTTTCACT	CGGGCTGAGC	TGGACAGAGA	GGATTTTGAG	1740

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FIG. 3c.

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CACGTGAAGA ATAGCACGTA TGAAGCCCTC ATTATAGCCA TTGACTTCGG TTCTCCAGTT 1800  
GCTACTGGAA CGGGAACCTCT TCTACTGGTC CTCTCTGATG TGAATGACAA TGGCCCCATT 1860  
CCAGAACCTC GAAATATGGA CTTCTGCCAG AAAAACCAC AGCCTCATGT CATCAACATC 1920  
ATTGATCCAG ATCTTCCCCC CAACACATCT CCTTTCACAG CAGAACTAAC ACACGGCGCA 1980  
AGTGTCAACT GGACCATCGA GTACAATGAC CCAGCTCGTG AATCTCTAAT TTTGAAGCCA 2040  
AAGAAAACCT TAGAGTTGGG TGAATACTCA ATAAATCTCA AGCTCACAGA TAACCAGAAC 2100  
AAGGACCAGG TGACCACCCCT ATATGTGTTT GTGTGCGACT GCGAAGGTGT CGTCAACAGC 2160  
TGCAAGAGGA CGGCGCCTTA CGCCGAAGCA GGCTTGCAGG TTCTTGCCAT CTTGGGCATT 2220  
CTCGGAGGAA TCCTCGCTCT ACTAATCCTG ATTCTGCTGC TTCTGCTATT TGTTCCGAGG 2280  
AGAAGGGTGG TCAAAGAGCC CTTACTTCCC CCAGAAGATG ACACCCGGA CAATGTTTAT 2340  
TACTATGATG AAGAAGGAGG TGGAGAGGAG GATCAGGACT TTGACTTGAG CCAGTTGCAC 2400  
AGGGGCCCTGG ATGCTCGGCC TGAAGTGACT CGCAATGATG TGGCCCCAAC CCTCCTGAGT 2460  
GTGCCCCAGT ATCGGCCCCG CCCTGCCAAT CCTGATGAAA TTGGAACCTT TATTGATGAA 2520  
AACCTGAAGG CAGCGGACAC TGACCCTACT GCTCCTCCTT ATGACTCTCT GCTCGTGTTT 2580  
GACTATGAAG GAAGCGGTTT TGAAGCTGCT AGTCTGAGCT CCTTGAACCT CTCAGAGTCA 2640  
GACCAAGACC AGGACTATGA CTACCTGAAT GAATGGGGCA ATCGCTTCAA GAAGCTGGCG 2700

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FIG. 3d.

2760 GACATGTATG GAGGTGGCGA GGACGACTAG GGGACTTGAG ACAAATGAAG ATGAGTCCTT  
2820 ATACCATGTG GTAGAAAATG CGGAGGTGAC TGTTTTCAGC TCCCTTCATC TGAGAGGAAT  
2880 TTCTGGAGAA GAGAAAATGC ACAGTGATAT ATAGTTAGGA TAGTTAGGAT TTCTACTTTA  
2940 TAGATCTAAT CTGTGTGTTT GTTAGAACGA TTTTGACCTA TTCTTTGAAG CTTTTTTTTC  
3000 TTTCTTTTCAT CATTCTTTAA ATGGTGATGC TGTCCAAAAG ACCCCCCACA TGTTTATATT  
3060 TCAAAAGAAT AGCTAAAGCC TCCAGAAGGT TCTGCTAGCA ATTTCGAGAT TGCCTTATTG  
3120 ACTTGTCTCA TTTTTTTTAA GGAAGGTAGG GCTAAACTAC CCTATTGTGT TTGTGTGTGT  
3180 GTGTGTGTAT GTGTAAATTAT TTTTAAATTG TGTTCCTTTT TCTCCTATCA CTGCACCTGGT  
3240 GTCCCGTGTT CTAATAACCA CTCTTAACTC CTTCCTGAACT TACATTGCCT CAGACAGGAG  
3300 TTCTCTGCTG CAGAAATTAT TGGGCCCTTT CAGGATAAGA GACTTGGTCT TAGTTTGATG  
3360 GTAGTGTGAC TGGGTATTAT GGACTCGTAA GGACTTTAGT GGTTCCTCCT TTTTATTTCC  
3420 TAAGTACATA AATTGAAATT CATATCCATC CACTGACTTG TTCTGCATTA AGTGTGTTTG  
3480 TCATGTGGAC GTCAATTATTG GGCTACTTTG GTTCTGAACA AGGAGCATTG ACCAGAAAAG  
3540 GTGGTGAATT TTCAGGTGCC ACTCAACTTC TAATGTTTAC TTATCACTCA AACAGAAGAG  
3600 TGATCTATTG TGACGTTTAG CGTAGTGCCT GCAGTGCTGC AGCCAAAGAT TGAAGGCGGA

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TTGTCAAAGC CAAGGCAAC ATGAAAAATG GACTTGGAGG TGGCAGGCGG GATGGGTCAT 3660  
TGAGCCTGGC GTTTTAGCAA ACTGATGCTG AGGATAACTG AGGTGGCTCT ACCTCTAGTC 3720  
CTGAAAAATTC TGAAGAAATGG AAGAATCCCG ACAAGTGTGT CCTATCGCGA TCCTTAGGTC 3780  
ACAGTTTGTG CCTGAGGCCA AGAATCCCCA GGTGCCCTGCT TTTGTTAATG TCTACCGAAA 3840  
ATGCAGCCTG ATCTGGACTC AGGTGCCCCA ATTCTAAGTG TGCATAGAAA ACTGACAATA 3900  
TTAGGAAATTT CTTTTTCCTC CCTTAGGAGC AGGAAGAAA TATGACCCTA AAGGGTTTTC 3960  
GCAAGGGAA GGTGGGGAGA GCTTTGACTT GGATTTTTTT TAAATTGAAA TGTGAACTTC 4020  
AAGGAACTTT TGACAACCAT GGGAAATAAT TTTATCTTAA ATTGCTTTAC TGTCTGTCAG 4080  
CTGTTTTTC AAGAAAAAA AAATCATCCC TGCAATCACT TCCTTGAATT GTCTTGATTT 4140  
TTCAGCAATT TAAACTCTAA TTTAGTCCTG TATAGAGAAT GTTAATGTAG TTTTGAGTGT 4200  
ATATGTGTGT GGGTACGGAT AATTTGTAT TTTCTTTAGG TCTGGAAGA GAAAACAATT 4260  
TAAGCTGCGA AAATTCTTAA ATATTCATTT TTATAAAATTT TATTAAAGAA TTTTGTGTTAA 4320  
AAAAA AAA 4333

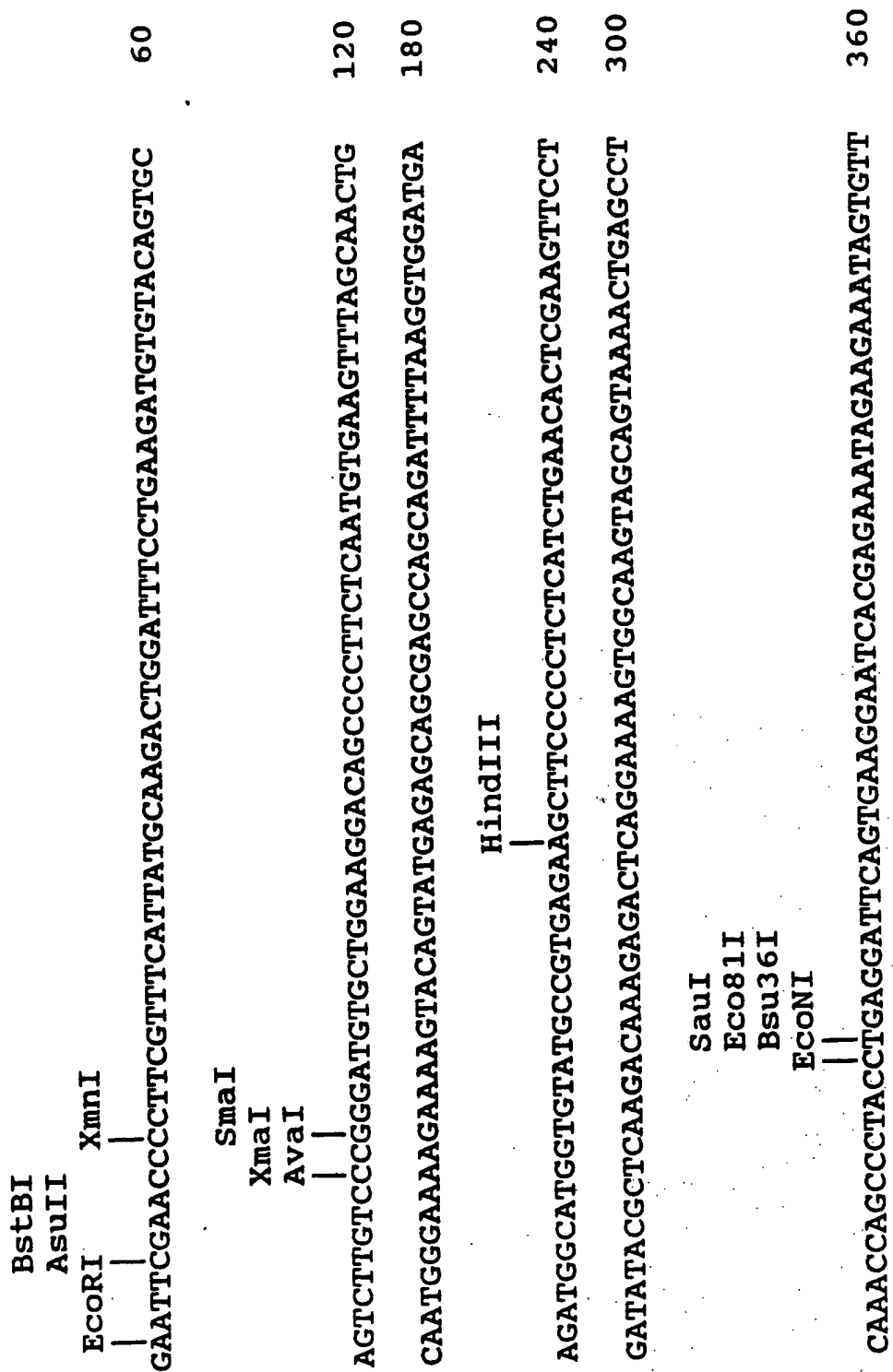
FIG.3e.

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FIG. 4a.

## N-cadherin restriction map



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FIG. 4b.

TCCAAGACAAGTGA	BspMI		
CTACTAAGCAACA	PstI		
ATGGCTACCTGC			
AGAGGCAGAGAG			
AGACTGGGTAT			420
CCCTCCCATCAAC	EcoO109	SstI	
TTGCCAGAAAAC	EaeI	SacI	
CTCCAGAGGGCC	DraII	HgiAI	
CTTTTCCTCAAG		Bsp1286	
AGCTCGTCAGG		BanII	
AT			480
CAGATCCGATAG	XhoII	AlwNI	
ATAAAACCTTTC			
TCTCTGCGGTAC			
AGCGTAACTGGG			
CCAGAGCTGA			540
CCAGCCTCCAAC		PvuII	
TCTTCATTATCA			
ACCCCATCTCAG			
GTGTCAGTAACCA			600
	BstXI	NspHI	
		Bsp1286	
		AseI	

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FIG. 4c.

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GCCTCTGGATCGTGAGCTGATAGCCCGGTTTCATTTTGAGGGCACATGCAGTGGATATTAA 660

Tth1111I

TGGAAACCAAGTGGAGAACCCCATCGACATTGTCATCAACGTTATTGACATGAATGATAA 720

SauI

Eco81I

Bsu36I

AlwNI

CAGACCTGAGTTCTTACACCAGGTTTGGAATGGGACAGTTCCTGAGGGATCAAGCCGGG 780

NdeI

AACATATGTGATGACGGTCACTGCGATTGATGCTGACGATCCAAATGCCCTCAATGGGAT 840

HaeII

BbeI

NarI

BanI

EcoNI AhaII

NspHI

AflIII

GTTGAGGTACAGAAATCCTGTCTCCAGGCGCCAAGCACCCCTTCGCCCAACATGTTTACAAT 900

PvuII

CAACAAATGAGACTGGGGACATTATCACGGTGGCAGCTGGACTTGACAGAGAAAAGTACA 960

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FIG. 4d.

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AccI | NdeI | 1020  
 ACAGTATACGTTAATAATTCAAGCTACAGACATGGAAGGCAATCCCACATATGGCCTTTC  
  
 HincII | BspMII | AccIII | 1080  
 CAACACAGCCACGGCTGTCATCAGGTGACAGATGTCAACGACAATCCTCCGGAGTTAC  
  
 TGCCATGACGTTCTATGGTGAAAGTCCCTGAAACAGGGTAGATGTCAATCGTCGCTAATCT 1140  
  
 Cfr10I | 1200  
 AACAGTGACAGATAAGGATCAGCCCCACACACCGGCTGGACGCCATCTACAGAATCAG  
  
 NaeI | Eco52I | 1260  
 CCGTGGAGACCCCGCGCGCTTTGCCATTCAAACTGACCCCAACAGCAACGCGGTTT  
  
 EagI | Cfr10I | 1320  
 AGTCACCGTAGTAAACCAATCGACTTTTGAAACAAATAGGATGTATGTCTTACTGTCCG  
  
 PstI | StyI | HincII | 1380  
 TGCAGAAAATCAAGTGCCATTAGCCAAGGGTATTCAGCATCCACCTCAGTCAACTGCGAC

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FIG. 4f.

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CACAGCACTTGATATGACATTGATCCAAATGCTGGACCAATTGCTTTTGATCTTCCTTT 1860  
 PflMI  
 GTCTCCAGTGAATAAGAGAAATTGGACCATCACTCGGCTTAATGGTGATTTTGCTCA 1920  
 CelII  
 GCTTAACCTTAAAGATAAAATTCTTGAGCCGGGATCTACGAAGTTCCAATCATAATCAC 1980  
 XhoII  
 AGATTGGGGTAATCCTCCCAAATCGAAATATCTCCATCCTTCGGGTGAAGGTTGCCAGTG 2040  
 Cfr10I  
 Bsp1286  
 Bani Bani  
 TGATTCCAACGGGACTGCACAGATGTGGATCGAATTGTGGGAGCAGGGCTGGGCACCCGG 2100  
 HaeII  
 BbeI  
 NarI  
 AhaII

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FIG. 4g.

CGCCATCATCGCCATCCTGTCTTGCATCATCATCCTGCTCATTCCTGTTCTGATGTTTCGT 2160  
GGTATGGATGAACGCCGGGATAAAGAACGCCAGGCCAAACAACCTTTTAATTGATCCAGA 2220

DraI  
SspI AhaIII  
|

AGATGATGTAAGAGATAATAATTTTAAATAATGATGAAGAAGGTGGAGGAGAAGAAGACCA 2280  
GGACTACGATTTGAGCCAGCTCCAGCAGCCCTGATACGGTAGAGCCAGATGCCATCAAGCC 2340

EaeI  
|

AGTTGGAATCCGACGGTTGGATGAGAGGGCCCATCCATCGGGAGCCCCAGTACCCGGTTCCG 2400

Bsp1286  
BanII  
|

PstI  
|

ATCTGCAGCCCCACACCCAGGGGACATCGGGGACTTCATTAAATGAGGGCCCTTAAAGCTGC 2460  
TGACAAAGATCCCACCGCTCCGCCCTACGACTCCCTCTTAGTCTTTGACTATGAAGGCAG 2520

Eco0109  
EaeI  
|

AseI  
|

DraII  
|

SstI  
SacI  
HgiAI  
Eco0109  
Eco52I  
EagI  
|

Bsp1286  
BanII  
|

TGGCTCCACGGCCGGTCTTGAGCTCCCTTAAATTCCTCCAGTAGTGAGGTGAGCAGGA 2580

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FIG. 4h.

Bsp1286  
 BanII  
 ApaI  
 EcoO109  
 DraII  
 EcoO109  
 EaeI  
 DraII  
 |||  
 CTATGACTATCTGAACGACTGGGGGCCCGCTTCAAGAACTCGCTGACATGTACGGTGG 2640  
 AGGTGATGACTGAACTTCAGGGTGAACTTGCTTTTGGACAAGTACAAACAATTGCAACT 2700  
 BsmI  
 |  
 GATATCCC AAAAGCATT CAGAAGCTAGGCTTTAACTTTGTAGTCTACTAGCACAGTGC 2760  
 ACCI  
 |  
 TTGCTGGAGGCTTTGGCAGAGGCTGCAAACCAATTGGGCTCAGAGGGAATATCGGTGAT 2820  
 Bsp1286  
 BanII  
 |  
 AlwNI  
 |  
 SstI  
 SacI  
 HgiAI  
 Bsp1286

FIG. 4i.

BanII  
 CCAATACTGTTGGAAAACACTGAGCTCAGTTACACTTGAAATTTTACAGTACAGAAGCAC 2880  
 TGGGATTTATGTGCCCTTTTGTACCTTTTTCAGATTGGAAATTAGTTTATGTTTAAGGC 2940  
 SspI  
 TTTAATGGTACTGATTTCTGAAATGATAAGTAAAGACAAAATATTTTGTGGTGGGAGCA 3000  
 GTAAAGTTAAACCATGATATGCTTCGACACGCTTTTGTGTACATCGCATTGTGCTTTTATTAA 3060  
 styI  
 AAATATGGAATTAAACAGACAAACCAACCCTCATGGAGCAATTTTATTACCTTGGGGGC 3120  
 BstXI  
 TGAGACCATGAGATTGGAAAATGTACATTATTTCTAGTTTTAGACTTTTAGTTTCTTGTTT 3180  
 PvuII  
 TGTTTTTTTCCACTAAAATCTTAAACTTACGCAGCTGGTTGCATAAAGGGAGTT 3240  
 XmnI  
 TTCAATACCAATTTGTAGCAAAAATTGAATTTTTCATAAAGTATGTTAGACACAT 3300  
 TTTGGTCTTAAATCCATGTACACTTTTATTATTACTGTATTTTTCACCTTCACTGTAAAA 3360  
 ATGGTATGTGTACATAATGTTTATTATGGCATAGTCTATGGAGAGTGCAGAAACTTCAGA 3420

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FIG. 4j.

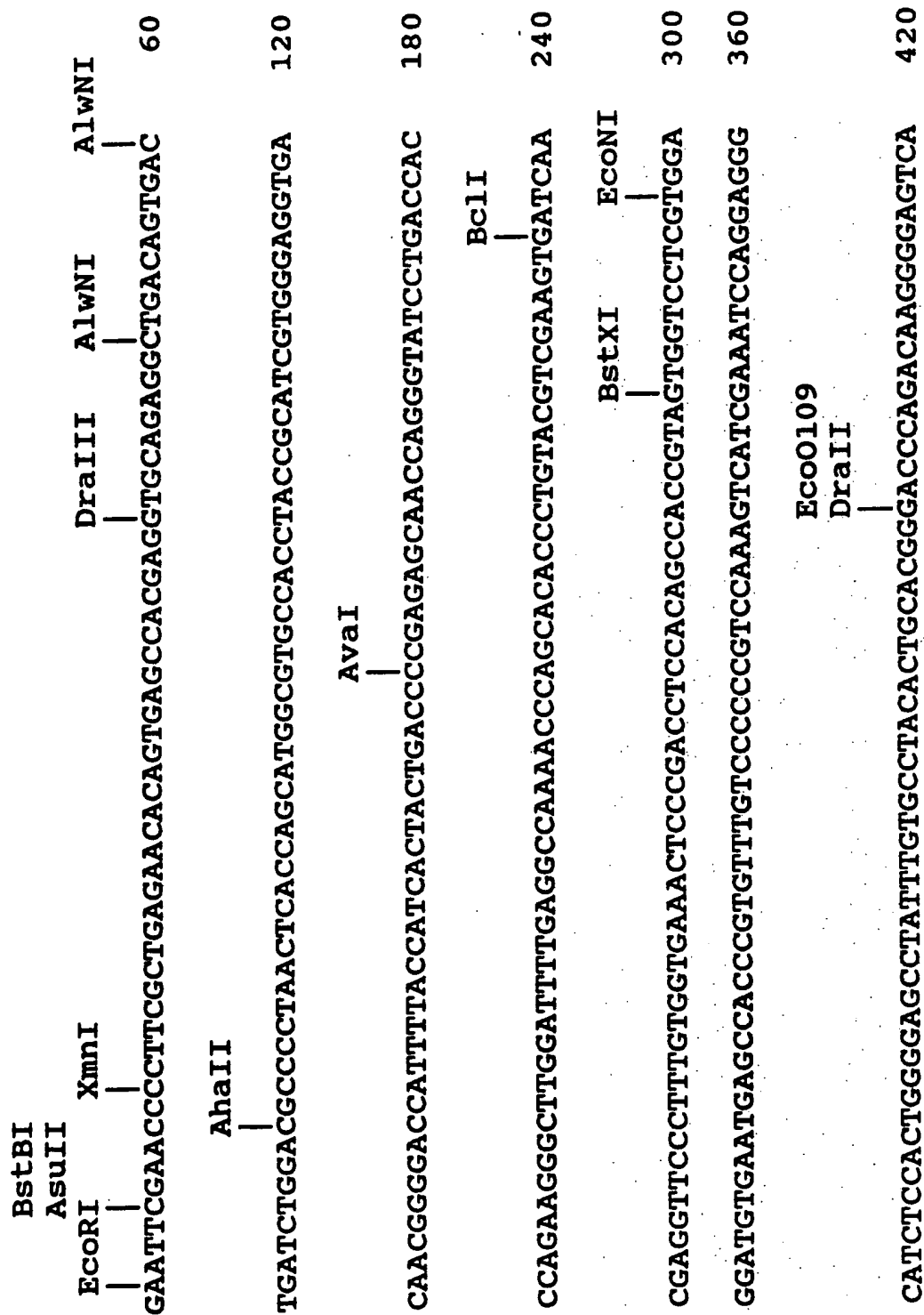
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NspHI AflIII 	NspHI 	
ACATGTGTATGTATTATTGGACTATGGATTCAGGTTTTTTTGGCATGTTTATATCTTTCGT		3480
TATGGATAAAGTATTTACAAAACAAAGTGACATTTGATTCAATTGTTGAGCTGTAGTTAG		3540
AATACTCAATTTTAAATTTTAAATTTTATTTTATTTTCTCTTTTGTGTTGGGG		3600
AGGGAGAAAAGTTCTTAGCCACAAATGTTTACATAATTGTACCACAAAACAAAAA	BstEII 	3660
AAAGGAAAGACAAAGAAATGAAAGGGTGACCTGACACTGGTGCTACTACTGCAGTGTGTG	PstI 	3720
DraI AhaIII 	DraI AhaIII HindIII 	
TTTTTAAAAAAATGAAAAAAAGCTTTTAAACTGGAGAGACTTCTGACAAACAGCT		3780
TTGCCCTCTGTATTGTGTACCAGAAATATAAATGATACACCTCTGACCCCGCGTTCTGAAT		3840
AAAATGCTAATTTTGGAAAAAATAAAAAA		3875

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FIG. 4k.

## P-cadherin restriction map



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FIG. 4I.

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	NheI		PflMI	
	BstXI			480
GAAGATCAGTTACCA				
CATCCTGAGAGACCC				
AGAGCCAGGGTGGCT				
AGCGATGGACCCAG				480
TGGACAAGTCACTG				
CCCGCAGGGGTCTT				
GGACCGTGAGGATG				
AGCAGTTTGTGAGA				540
AAACAA				
	Bsp1286			
	BanII			
CATCTACGAAGTCA				
TGGTCTTGGCCACA				
GATGATGGAGCCCT				
CCCCACCACTGGCA				600
CAGG				
	EcoO109			
	DraII			
GACCCCTCCTGCTA				
ACA				
CACTGATGGACATCA				
ATGACCA				
CGGTCCCGGTCCCG				
GAGCCCCCGTCA				660
	Bsp1286			
GATCACCATCTGCA				
ACC				
AAAGCCCTGTGCCC				
CCAGGTGCTAAACA				
TATCACA				
GAGCAAGGACTT				720
	EaeI			
GTCCCCCACA				
CTGCCCCCTTTC				
CAGGCCCCAACTCA				
CACATGACTCGGAC				
GTCTATTGGAC				780
	HincII			
AGCAGAA				
GTCAACGAGAAAGG				
AGAGACGAGTAGCC				
CTTGTCCCTGAAGA				
AGGTTCTCTAAAGCA				840

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FIG. 4m.

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HgiAI  
 Bsp1286  
 ApaLI  
 AGCGGAATACGATGTGCACCTTTCCCTGTCCGACCACGGCAACAAGGAACAGCTGACAGT 900  
 PvuII  
 BclI  
 DraIII  
 BstEII  
 BspMI  
 EcoO109  
 DraII  
 GATCAGAGCCACCGTGTGTGACTGCCACGGCAACATGGTGACCTGCCGGGACCCCTGGAC 960  
 GTGGGGTTTCCTCCCATCCTGGGGTGCTGCCCTGGCTCTGTGCTCCTTCTGTGCTGGT 1020  
 HgiAI  
 Bsp1286  
 GCTCCTATTCTTGGTGAGAAAGAAACGGAAGATCAAGGAACCCCTTCTCCTCCCAGAAGA 1080  
 XmnI  
 Tth111I  
 TGATACCCGTGACACGCTTCTACTACGGCGAAGAGGGGGTGGCGAGGAGGACCAGGA 1140  
 SauI  
 Eco81I  
 Bsu36I  
 EaeI  
 CTATGACATCACCAGCTCCACCGGGTCTGGAGGCCCGCCCTGAGGTGGTTCTCCGCAA 1200

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FIG. 40.

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BspMI  
 |  
 CTC A A G G G C A G G T C T C T A T G C C T T T C A G A A C G G A G G A A C C G T G G G C A G T T T G A T T T C A A 1680

HgiAI  
 Bsp1286      EcoNI  
 |  
 C A G T G A G C A C C T C T T A G C C T A A G C C A G G G C T G C T C A A T T T C T G G A G T C T C C T C G C T A C C 1740

EcoO109  
 DraII  
 Eco47III  
 CeliI    HaeII  
 |    |    |  
 A T A A A T G C T C A G C G C T G G G T C C T G G G T T T G A C T G A C T G A C T T T C C C A T G A T G G C T T 1800

StuI  
 EaeI  
 |    |  
 T T G C T C T G G A A T G G A C C C T T C T C C T T A G T A A C A G G C C T C T T A C C A C A A T C T T C G T T T T T 1860

EcoO109  
 DraII  
 BspMI  
 |  
 T T T T T T T A A T G C T G T T T T C A A A A G T G A G A G G C A G G T C C T C A A C C A C C C C C T G G A G C G C T 1920

Bsp1286    NsiI  
 |  
 C C A G A G C C C A G G C G T G C C C T C A T G C A T T T C T C T G T G G T C T C T T G G C C C C C A G A C C T C C T 1980

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[illegible]

## E-cadherin restriction map

BanI  
 |  
 CGGGCACCTGTGATTCCGGGAAGTCCTGCCGCCCTCGCGCCGCTCGCGCCCGGCTCTCGA 60  
  
 BanII HaeII  
 ApaI BbeI  
 EaeI NarI  
 StyI EcoO109 AhaII  
 NcoI DraII Bani  
 | | | |  
 CCCCCCGCCCATGGGCCCTCGGTACGGGGCGCCCCCGGCTCCTGCTCCCGCTGCTG 120  
  
 BspMI PstI BanII BglI  
 | |  
 CTGCTGCTGCAGGTCTCATCGGGGCTCTGCCAAGAGCCGGAGCCCTGCGCCCGCTGGCTTT 180

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[illegible]

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FIG. 4r.

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styI  
 |  
 CAAGGAGCTGACGCACCTCCTGTGTGTGTTTATTATTGAAAGAGAAACAGGATGGCTG 720  
  
 AAGTGACTGAGCCCTCTGGATAGAGAAACAAATTGCTAAGTACATTCTCTACTCTCATGCC 780  
  
 GTATCTTCTAATGGGAATGCGGTTGAAGACCCCAATGGAGATCGTGATCACGGTGACAGAT 840  
  
 CAGAAATGACAAAGCCCGAGTTCACCCAGGCAGTCTTCCAAGGATCTGTCAACGGAAGGT 900  
  
 GCCCTTCCAGGCACCTCTGTGATGCAGGTGACAGCCACAGATGCGGATGATGTGAAT 960  
  
 ACCTACAACGCTGCCATCGCTTACAGCATCCTCACACAAGACCCCTCCTGCCTAGCAGC 1020  
  
 ATGATGTTCACTATCAACAAGGACACAGGAGTCATCAGCGTGCTCACCACCTGGGCTGGAC 1080  
  
 CGAGAGGGTGTCCCCATGTACACCTTGGTGGTTTCAGGCTGCTGACCTGCAAGGCCGAAGGC 1140

BsmI  
 |  
 BclI  
 |  
 XhoII  
 |  
 Aval  
 |  
 StyI  
 |  
 BspMI  
 |  
 HgiAI  
 |  
 BstXI  
 |  
 StyI  
 |  
 BspMI  
 |

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FIG. 4s.

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BclI PvuII	AlwNI	
TTAACTA	CTGCAACAGCTGTGATC	ACAGTCACTGACATCAATGATAACCCCCCATC 1200
BamI		
TTCAACCC	AACCGTACCAGGACGGTGCTGCCGTGAGAACAAAGGCTAACGTCGAAATCGCT 1260	
	BglI	
GTACTCAA	AGTGACGGATGCTGATGTCCCCGATACCCCGGCTGGAGGGCTGTGTACACC 1320	
BclI		
ATATTGA	ACAATAACAATGATCAATTTGTGTGTCCACACAGACCCAGTAACTAACGACGGC 1380	
ATTTTGA	AAACAACTAAGGGCTTGGATTTTGAGGACAAGCAGCAGTATGTCTTGTACGTG 1440	
	AlwNI	
ACTGTGT	GAAACGTGACCCCGTTTGAGGTGATCCTCTCCACCTCCACAGCCACTGTCACT 1500	
GTGGACG	TGGAAGATGTGAATGAAGCCCCCATCTTTCATCCCTTGCCCAAAGGTAGTGTC 1560	
	XhoII BamHI	
ATCCCTG	AAGACTTTTGGTGTGGGCCAGGAAATCACATCCTACACCGCCGAGGATCCAGAT 1620	
	Cfr10I	



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FIG. 4t.

ACATATATGGAACAGAGGATAACGTATCGGATTTGGAGGGATGCTGCCGTTGGCTGGAG	1680
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>BanI</p> <p>PfIMI</p> <p>AlwNI</p> </div> <div style="text-align: center;"> <p>AvaI</p> <p>CellII</p> </div> </div>	
GTAAATCCAGAAATCTGGTGCCATTTTCACTCGGGCTGAGCTGGACAGAGGATTTTGAG	1740
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>HgiAI</p> </div> </div>	
CACGTGAAGAAATAGCACGTAATGAAGCCCTCATATAGCCATTGACTTCGGTCTCCAGTT	1800
GCTACTGGAACGGGAACCTCTTCTACTGTGTCCTCTCTGTGATGTGAATGACAAATGGCCCCATT	1860
CCAGAACCTCGAAATATGGACTTCTGCCAGAAAACCCACAGCCTCATGTTCATCAACATC	1920
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>XhoII</p> <p>BglII</p> </div> </div>	
ATTGATCCAGATCTTCCCCCAACACATCTCCCTTCACAGCAGAACTAACACACGGCGCA	1980
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>HincII</p> </div> </div>	
AGTGTCAACTGGACCATCGAGTACAATGACCCAGCTCGTGAACTCTAATTTTGAAGCCA	2040
AAGAAAACCTTTAGAGTTGGGTGACTACAAAATAAATCTCAAGCTCACAGATAACCAGAAC	2100
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>BstEII</p> </div> <div style="text-align: center;"> <p>PvuII</p> <p>HincII</p> </div> </div>	
AAGGACCAGGTGACCACCCTATATGTGTGTGTCGCGACTGCGAAGGTGTCGTCACACAGC	2160

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FIG. 4x.

	PstI	PstI	
TGATCTATTCTGACGTTTAGCGTAGTGCCCTGCAGTGCTGCAGCCAAAGATTGAAGCGGA			3600
StyI			
TTGTCAAAGCCCAAGGCAACATGAAAAATGGACTTGGAGGTGGCAGCGGGATGGGTCAT			3660
TGAGCCTGGCGTTTTAGCAAACTGATGCTGAGGATAACTGAGGTGGCTCTACCTCTAGTC			3720
		SauI	
		Eco81I	
		NruI	
		Bsu36I	
CTGAAAAATTCTGAAGAAATGGAAGAAATCCCGACAAGTGTGTCCTATCGCGATCCTTAGGTC			3780
	SauI		
	Eco81I		
	Bsu36I		
ACAGTTTGTACCTGAGGCCCAAGAAATCCCGAGGTGCCCTGCTTTTGTAAATGCTACCGAAA		BanI	3840
		AccI	
	BanI		
ATGCAGCCTGATCTGGACTCAGGTGCCCCCAATTCTAAGTGTGCATAGAAAACTGACAATA			3900
		SspI	
	SauI		
	Eco81I		
	Bsu36I		
TTAGGAAATTCTTTTCCCCCTTAGGAGCAGGAAGAAAAATATGACCCCTAAAGGGTTTG			3960

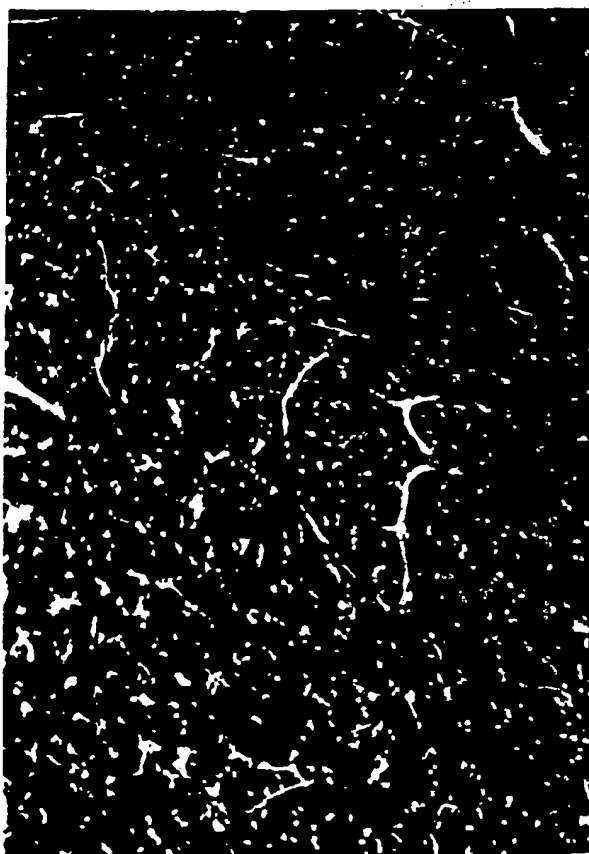
DraI  
 AhaIII  
 |  
 GCAAAGGGAAGGTGGGAGAGAGCTTTGACTTGGATTTTTTTAAATTGAAATGTGAACCTC 4020  
  
 StyI  
 NcoI  
 |  
 AAGGAACTTTTGACAACCATGGGAAATAATTTTATCTTAAATTGCTTTACTGCTGTCAG 4080  
  
 PvuII  
 |  
 CTGTTTTTCAAAGAAAAAAATCATCCCTGCAATCACTTCTTGGAATGTCTTGATTT 4140  
  
 DraI  
 AhaIII  
 |  
 TTCAGCAATTTTAAACTCTAATTTAGTCCCTGTATAGAGAAATGTTAATGTAGTTTGTAGTGT 4200  
  
 ATATGTGTGGGTACGGATAATTTTGTATTTTCTTTAGGCTGGGAAAAGGAAAACAATT 4260  
  
 SspI  
 |  
 TAAGCTGCCGAAAAATCTTAAATATTCAATTTTATATAAAATTTTATTAAAGAAATTTTGTGTTAA 4320  
  
 AAAAAAAAAAAAAA 4333

**FIG. 4y.**

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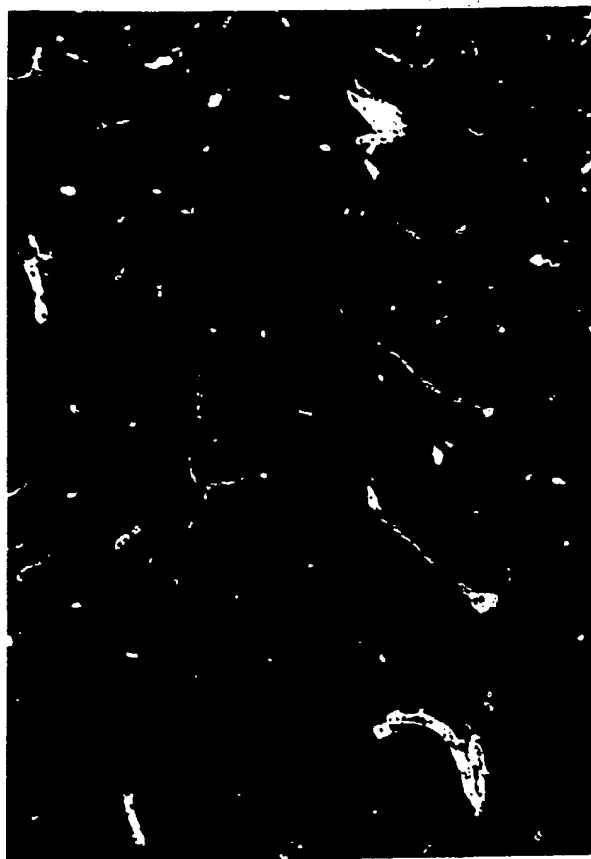
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FIG. 5.



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FIG. 6.





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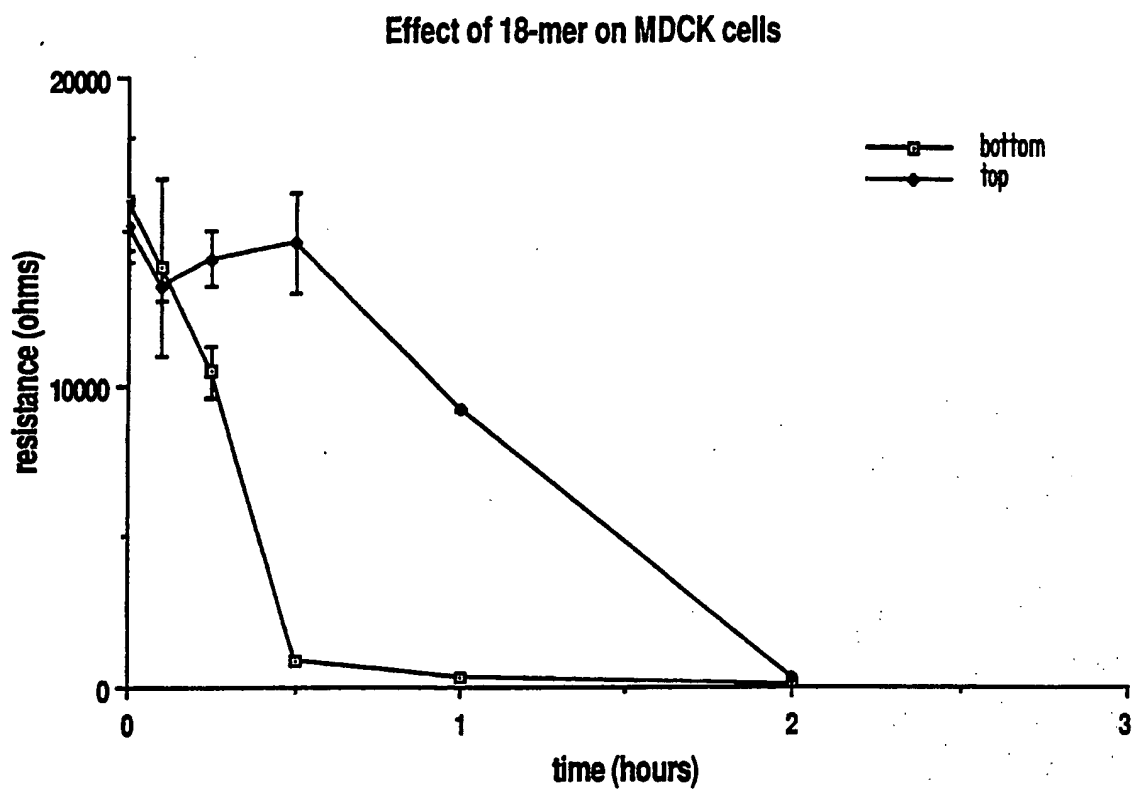


FIG. 7.

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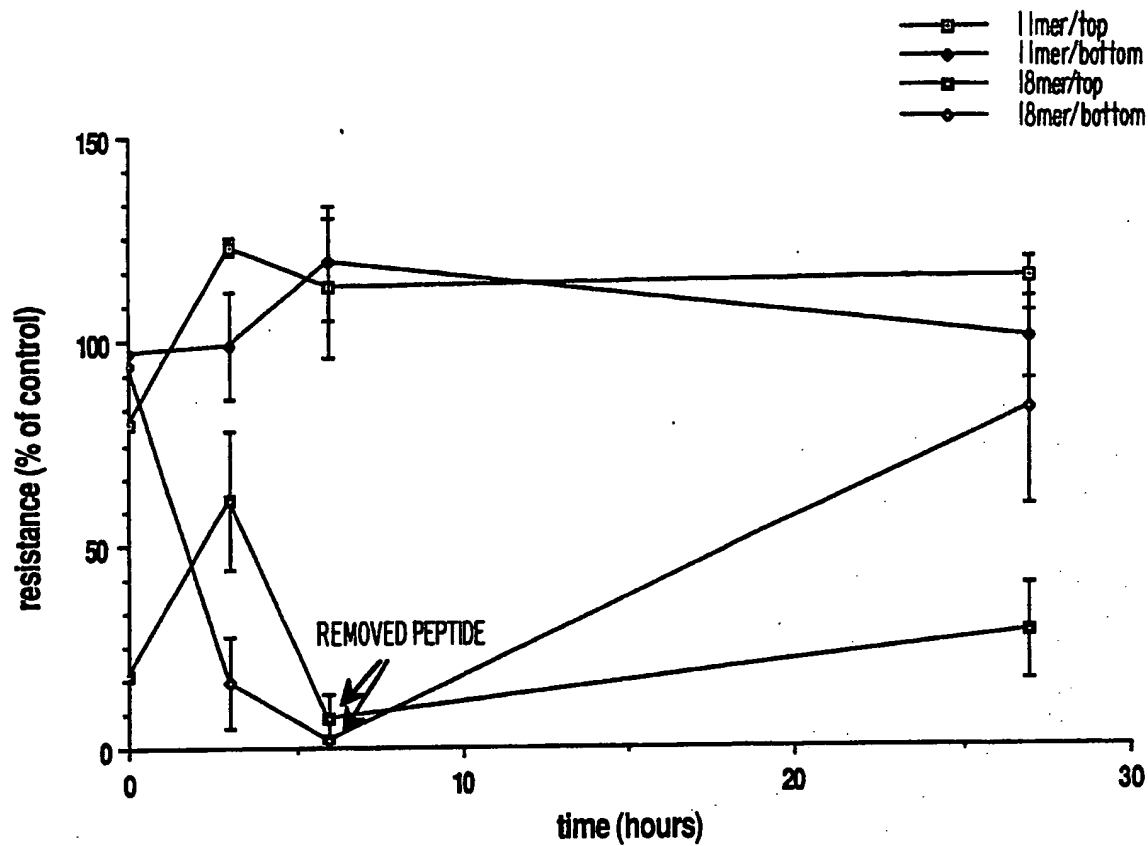


FIG. 8.

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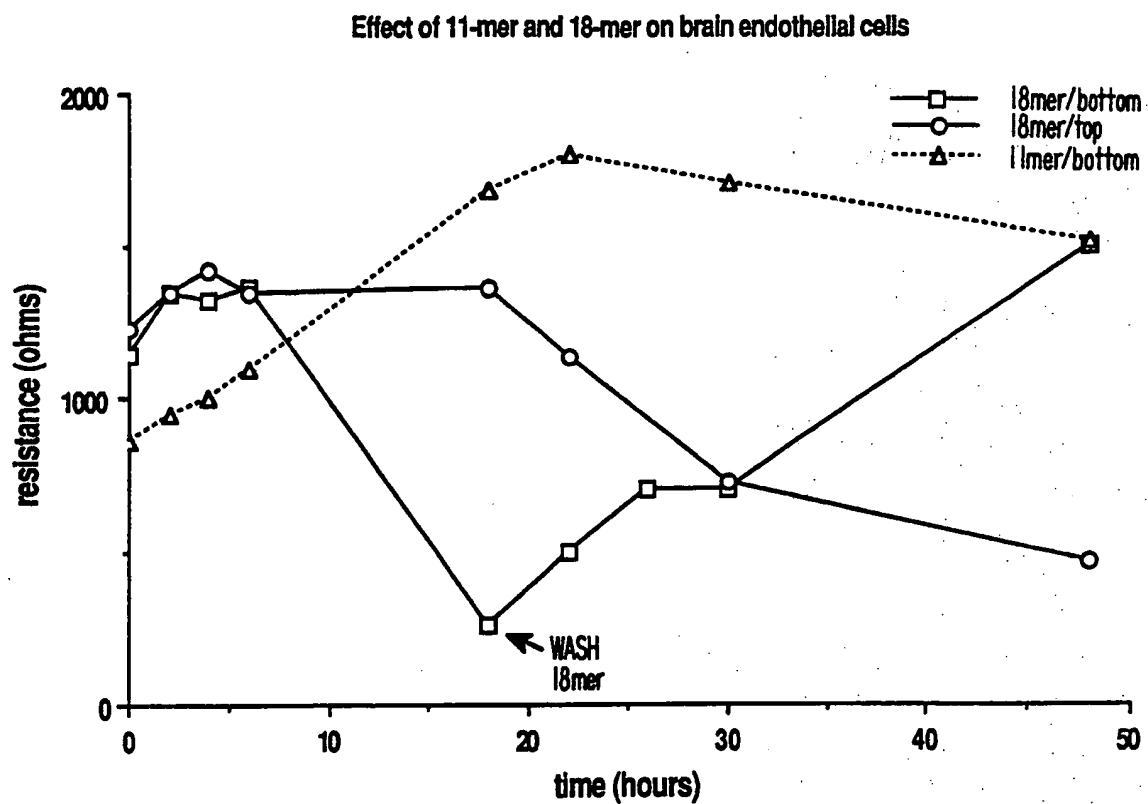


FIG. 9.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05105

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02, 39/00; C07K 7/08, 7/10, 13/00, 15/00, 15/28

U.S. CL.: 530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387; 514/12, 13; 424/85.8, 85.91

## II. FIELDS SEARCHED

### Minimum Documentation Searched \*

Classification System :

Classification Symbols

530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387  
514/12, 13  
424/85.8, 85.91

U.S. CL.

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched \*

Data bases: Dialog (Files; Medline, Biosis, Chemical Abstracts, World  
Patents Index) Automated Patent Searching (1975-1990)

## III. DOCUMENTS CONSIDERED TO BE RELEVANT \*\*

Category *	Citation of Document, <sup>1</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
<u>X</u> Y	The EMBO Journal, Volume 4, No. 13A, issued December 1985, Vestweber et al., "Identification of a Putative Cell Adhesion Domain of Uvomorulin," pp. 3393- 3398, See the Abstract and Discussion.	1-6,14-21,23-27 & 35-42  1-65
Y	Development, Volume 102, issued April 1988, M. Takeichi, "The Cadherins: Cell-cell Adhesion Molecules controlling Animal Morphogenesis," pp. 639-655 see the Summary and pages 643, 645 and 651.	1-65
<u>X</u> Y	The Journal of Cell Biology, Volume 107, issued October 1988, B. Gumbiner et al., "The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex," pp. 1575-1587 see the Abstract.	1-6,14-21,23-27, 35-42  1-6,14-27,35-47, 55-65

\* Special categories of cited documents: <sup>13</sup>

"A" document defining the general state of the art which is not  
considered to be of particular relevance

"E" earlier document but published on or after the international  
filing date

"L" document which may throw doubts on priority claim(s) or  
which is cited to establish the publication date of another  
citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or  
other means

"P" document published prior to the international filing date but  
later than the priority date claimed

"T" later document published after the international filing date  
or priority date and not in conflict with the application but  
cited to understand the principle or theory underlying the  
invention

"X" document of particular relevance; the claimed invention  
cannot be considered novel or cannot be considered to  
involve an inventive step

"Y" document of particular relevance; the claimed invention  
cannot be considered to involve an inventive step when the  
document is combined with one or more other such docu-  
ments, such combination being obvious to a person skilled  
in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search \*

Date of Mailing of this International Search Report \*

21 November 1990

04 FEB 1991

International Searching Authority \*

Signature of Authorized Officer <sup>19</sup>

*R. Keith Baker*  
R. Keith Baker, Ph.D.

ISA/IS

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, <sup>1a</sup> with indication, where appropriate, of the relevant passages <sup>1b</sup>	Relevant to Claim No. <sup>1c</sup>
Y	The EMBO Journal, Volume 6, No. 12, issued 1987, M. Ringwald et al., "The Structure of Cell Adhesion Molecule Uvomorulin Insights into the Molecular Mechanism of Ca <sup>++</sup> -dependent Cell Adhesion," pp3347-3353, see pages 3647-3648.	1-13,22-34,43-54 and 63-65
Y	US, A, 4.671,958 (Rodwell et al.) 09 June 1987, see the Abstract and Column 7.	43-47 and 55-65
Y,P	Development Biology, Volume 139, No. 1, issued May 1990, O.W. Blaschuk et al., "Identification of a Cadherin Cell Adhesion Recognition Sequence," pp227-229, see the entire Document.	1-65

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers \_\_\_\_\_, because they relate to subject matter<sup>1</sup> not required to be searched by this Authority, namely:
  
2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>1</sup>, specifically:
  
3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

**VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>**

This International Searching Authority found multiple inventions in this international application as follows:

**See Attachment**

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. **Telephone Practice**
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
  
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
  
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

**Remark on Protest**

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

Attachment To PCT/ISA/210  
Observations Where Unity Of Invention Is Lacking

Group I, claims 1-13 and 22-34, drawn to a composition for opening tight junctions and a method of use, classified in classes 530 and 514, subclasses 324, 326, 350 and 12 and 13, respectively.

Group II, claims 14-21 - 35-42, drawn to antibodies for opening tight junctions and methods of use, classified in classes 530 and 424, subclasses 387 and 85.8, respectively.

Group III, claims 43-54 and 63-65, drawn to a conjugates of a drug and a cell adhesion inhibitor, classified in class 530, subclasses 402, 409, and 345.

Group IV, claims 55-62, drawn to a conjugate of a drug and an antibody, classified in classes 530 and 424, subclasses 389, 390, 391 and 85.91, respectively.

Attachment To PCT/ISA/210

Detailed Reasons For Holding Lack Of Unity Of Invention:

PCT Rule 13.2 permits claims to "a" (one) product, "a" (one) method of making and "a" (one) method of using said product. The invention as set forth in Group I constitutes a combination of a product and a method of use. Groups II, III and IV one drawn to products that are distinct from that of Group I. Each of the products have a different structure and are distinct compositions as evidenced by their separate classification.